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Genetic aspects of a small scale honeybee breeding program

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Genetic aspects of a small scale honeybee breeding program

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Extensive contributions were also made by Bangor University and Tropical Forest Products Ltd. The West Wales Bee Breeding program was set up as a partnership between the University and Tropical Forest, one of Wales' largest bee farmers and importers of organic African honey and beeswax, based in Ceredigion. The ultimate goal of the project is be to produce a hardy, productive, strain of bees resistant to *varroa* and other diseases without the use of medications.

I'r Hogiau

Cofiwch

'Dyfal donc a dyrr y garreg'

Summary

Beekeepers in Wales, like others across the northern hemisphere, continue to experience high overwintering colony losses. Breeding for local adaptation has been recommended as part of the solution. The West Wales Bee Breeding Program (WWBBP) was therefore established in an effort to improve, through selection, the resilience and production potential of a local bee stock. Breeding for desired character traits began in 2011 and focused mainly on colony strength, *varroa* mite infestation, and temperament. Foraging efficiency was also monitored when conditions allowed. This thesis presents data from the first two rounds of selection. Scant evidence indicating adaptive change due to selection was detected across this time frame, but a demonstrable reduction in the variance of colony strength was observed.

The influence of selection across generations on population level genetic variation was also monitored. Microsatellite loci were highly polymorphic in the source population, and great diversity was also observed at a custom *csd* marker. Low frequency alleles at both marker types were lost across generations, and a significant difference in allelic richness was observed between the source population and each of the following two daughter generations. The effects of various selection/breeding parameters on the rate of genetic depletion due to selection within a contemporary timeframe (5 generations) were simulated, and the possible consequence of long term genetic depletion on adaptive response was considered. Simulations indicated that the number of breeder queens selected had the greatest influence on the rate of genetic depletion at both neutral loci and at the *csd* locus, across years.

The WWBBP aims to enhance local suitability through selective breeding while concurrently preserving genetic diversity and adaptive potential in the simplest most practical way. Hopefully, this thesis will help guide the future development of the program, and in addition, provide a basic transferable template for successful small-scale breeding.

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Glossary

Chalkbrood- A fairly common fungal brood disease caused by the *Ascosphaera apis.* Its effect on the majority of hives is only slight but it can adversely affect small colonies in the early spring.

Grafting- First performed by G.M. Doolittle, and described in his book Scientific Queen Rearing, published in 1888. It is the process of artificially raising queens by removing larvae of appropriate age (from a colony of choice) and placing them in artificially made (beeswax or plastic) cell cups. Many larvae can in this way be presented to a prepped queenless cell raising colony. Strong cell raising colonies can raise up to 100 or more cells under optimal conditions.

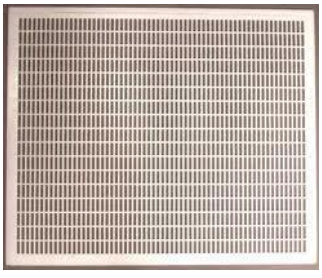
Nucleus- Nucleus colonies are small colonies that are created from larger colonies. The name is derived from the fact that a nuc hive is centered around a queen - the nucleus of the honey bee colony.

Split- A term used to describe the process of 'splitting' a large colony into two or three separate colonies, each with equal amounts of brood and stores. The original will retain the queen, while the others may be left with brood and bees of appropriate age to raise a new queen. A 'walk-away' split is one way beekeepers use to expand their operation.

Spotty-brood- This is a characteristic brood pattern that results from the removal of diploid drones by workers in a colony headed by a poorly mated queen.

Supersedure- This is the process of naturally replacing an existing queen. Bees can sense when an old queen is failing and will raise a replacement.

Queen Excluder- A plastic or metal grid that allows workers to pass through but restricts the movement of drones and queens. It is commonly used to prevent queens from moving vertically in the hive.



Queenright- Colony has a queen

csd Complementary sex determination

H_e	Expected heterozygosity
H_o	Observed heterozygosity
M_e	Effective mating success
N_b	Parental contribution from previous year
N_e (chapter 2)	Estimated mating success
N_e (chapter 4)	Effective population size
N_o	Observed mating frequency
h^2	heritability
V	Brood viability
V_P	Phenotypic variance
V_G	Variance due to genetic effects
V_E	Variance due to environmental effects
V_A	Variance due to additive genetic effects

Chapter 1

General Introduction

1 *Introduction*

The Western honeybee, *Apis mellifera* (Hymenoptera, Apidae) is an old and highly successful species. The development of colony life relaxed environmental constraints allowing honeybees to expand across a broad range of climatic and ecological conditions (Moritz et al., 2005). It adapted to arid sub-tropical conditions in the south, to cold temperate conditions in the north, and its range extends across Western Europe from the Atlantic coast of the Iberian Peninsula, to the Ural Mountains in the East. Correspondingly diverse ecotypes evolved against this broad ecological background and there are currently 24-26 recognized ecotypes or subspecies (De la Rua et al., 2001; Moritz et al., 2005). Morphological comparisons by F. Rutter, later supported by genetic analyses (Garnery et al., 1992; Franck et al., 1998), collapse these sub-species into four distinct lineages (M, O, A and C). Recent analyses of whole genome data propose an alternative to the previously accepted hypothesis that the honey bee radiation initiated in Asia, suggesting instead, two possibly separate out of Africa expansions and subsequent radiations (Whitfield et al., 2006).

1.1 *Ecological and Economic role*

Honeybees play a critical role as angiosperm pollinators, and are of vital economic and ecological importance (Genersch et al., 2010). Certain aspects of their biology make them well suited for this purpose. They are generalists, able to forage and thrive on a wide range of nectar and pollen sources, and to travel long distances to do so. Bees employ complex communication behavior to pass information relating to location of nectar sources. They are well suited to pollinate commercial crops. Thirty five percent of the food consumed by people is pollinated by animals (Genersch et al., 2010), and the large-scale homogenized agriculture practiced in Europe and the US requires pollination services from managed honeybee apiaries. California exported $2.3 billion worth of almonds in 2010 alone, a crop that is dependent upon the pollination services of honeybees. It is also claimed that honeybees contribute between £200 million (British Beekeepers Association), and bees in general up to £430 million pounds per annum (UK National Ecosystem Assessment) to the British economy. Honeybees are responsible for pollinating a range of crops and are responsible for pollinating 90% of the UK's apple crops.

1.2 *Honeybee Health and Disease*

Honeybees live close social lives. They not only associate intimately with other members of the colony, but are part of a community of organisms that may interact in beneficial, neutral or antagonistic ways. They are susceptible to damage from a wide range of metazoan, microbial and viral pathogens. Antagonists include: mites and beetles (*Varroa* and *Acarapis* mites, and small hive-beetle); Microsporidia (*Nosema apis* and *N. ceranae*) and other fungi; bacteria (American and European foulbrood); and viruses.

The bee population dramatically crashed in America over the winter of 2006-2007. These collapse events were characterized by the sudden disappearance of adult bees, and with apparent abandonment of hives, brood and food resources (vanEngelsdorp and Meixner, 2009). These symptoms collectively define colony collapse disorder (CCD), a newly described specific collapse syndrome. Seasonal losses among managed colonies have remained high since 2008. Preliminary survey results indicate that 31.1% of managed honey bee colonies in the United States were lost this winter (2012/2013) (vanEngelsdorp et al., 2013) and there was a critical shortage of bees for pollination on the almonds. Although bumper crops are still expected (estimated to be over 2 billion pounds) due to very good growing conditions, there is growing concern that ever diminishing bee numbers may provide a problem for growers in the future.

Although CCD is recognized as a syndrome specific to North America, similar declines in bee colonies were experienced in Europe. In France, the death rate was more than 60% and England lost 30% of its colonies over the winter of 2007-2008 (Aston, 2010). No single causative agent has yet been found. Worldwide incidents of unusually high levels of colony deaths or "disappearance diseases" have been periodically reported (Table 1.1). There have been 18 major episodes since 1869 (Underwood and vanEngelsdorp, 2007). An infamous epidemic occurred in Britain during the early years of the 20th century. No causative agent for the 'Isle of Wight disease' was isolated during the outbreak, and by 1919, Britain had lost 90% of its colonies. The microsporidian, *Nosema apis*, was subsequently highlighted as a possible cause, as was the tracheal mite *Acarapis woodi* (Neumann and Carreck, 2010). Chronic paralysis virus (CPV), identified in diseased Isle of Wight bee samples by Lesley Bailey in the 1950's, is now considered to be the most likely cause of the outbreak (Allen and Ball, 1996; Bailey, 1964).

Other 'disappearance' outbreaks occurred in United States and Canada around 1920, and again in the south and south western USA in the 1960's (Underwood and vanEngelsdorp,

2007). Outbreaks of so-called 'disappearing syndrome' occurred in Australia and 'disappearing disease' in Mexico in 1975, with environmental factors determined to be likely causes. Greater than average losses were reported in the United States during the end of the 1970's and again in the mid 1990's (Underwood and vanEngelsdrop, 2007). France experienced devastating losses between 1998 and 2000 with disease, stress due to poor nutrition and chemicals in the environment being presented as possible contributors. The cause is still not known.

Table 1.1 Historical large-scale colony losses

Year	Location	
1868	Kentucky, Tennessee	Anonymous, 1869
1872	Australia	Beuhne, 1910
1906	Isle of Wight	Bullamore, 1920
1910	Australia	Behune, 1910
1915	Portland, Oregon	Root and Root, 1923
1915	Florida to California	Tew, 2002
1917	United States	Root and Root, 1923
1917	New Jersey, Canada	Carr, 1918
1960's	Louisiana, Texas	Williams and Kauffeld, 1974
1963-64	Louisiana	Oertel, 1965
1964	California	Foote, 1966
1970	Mexico	Mraz, 1977
1970's	Seattle, Washington	Thurber, 1976
1974	Texas	Kauffeld et al., 1976
1975	Australia	Olley, 1976
1977	Mexico	Kulinčević et al., 1984
1978	Florida	Kulinčević et al., 1982
1995-96	Pennsylvania	Finley, 1996
1999-2000	France	Faucon et al., 2002
2002	Alabama	Tew, 2002
2002-2003	Sweden and Germany	Svensson , 2003

The honeybee is vulnerable to a wide range of threats including: habitat degradation, irresponsible pesticide use, genetic pollution, human-mediated pathogen translocation and climate change. Synergistic interactions between two or more of these antagonists can overwhelm susceptible bee populations (Neumann and Carreck, 2010). For example, the parasitic mite, *Varroa destructor*, has facilitated the decline of managed and native honeybee populations worldwide. It has a relatively benign association with its native host *Apis cerana*, but has demonstrated greater virulence in *A. mellifera*. *Varroa destructor* has been associated with viral transmission and immune system suppression in honeybees

(Cox-Foster, 2007). The significance of the association between *varroa* and deformed wing virus (DWV), and its influence on virus prevalence, load, and diversity, was recently highlighted by Martin et al. (2012). They investigated how *varroa* affected the spread of DWV in a newly colonized region (Hawaii in this case). They showed how the arrival of a DWV strain that can replicate in *varroa*, led to the rapid spread and dramatic increase in viral loads across the island. While the distribution and prevalence of other common viruses remained unaffected, *varroa* radically and rapidly shifted the DWV viral landscape.

Table 1.2. Approximate worldwide timeline for *Varroa destructor* (Acari: Varroidae) expansion

Location		Detected
Asia	Soviet Union	1960 Hatcher and Batty, 2011
	Philippines	1957 Navajas, 2010
Europe	Bulgaria	1972 Navajas, 2010
	Romania	1975 Hatcher and Batty, 2011
	Britain	1992 " "
North Africa	Libya	1976 Hatcher and Batty, 2011
	Tunisia	1975 " "
South America	Paraguay	1971 Hatcher and Batty, 2011
	Brazil	1975 " "
North America	United States	1987 Wenner and Bushing, 1996
	Hawaii	2007 Ramadan et al., 2007
Africa	South Africa	1997 Fazier et al., 2009
	Tanzania	2009 "
	Kenya	2009 "
New Zealand	New Zealand	2000 Goodwin and Van Eton, 2001

1.2.1 Varroa

Varroa destructor is an obligate ectoparasitic mite that has become a worldwide pest of the western honeybee, *Apis mellifera* (Gisder et al., 2009; Table 1.2). It evolved in concert with its native Asian host, *Apis cerana* (Moritz et al., 2005), and was first observed on western honeybees, *A. mellifera*, in Singapore in 1951. It now infests colonies on all

continents other than Australia. It was recently reported to be in East Africa, and is likely more widespread across the continent (Fazier et al., 2009). By examining sequence variation within the cytochrome c oxidase subunit 1 mitochondrial region (CO-I sequence variation) and by using morphological comparisons of mites from around the world, Anderson and Trueman (2000) demonstrated that *V. destructor* is part of a two-species 'complex' comprising of *V. destructor* and *V. jabobsoni*. *Varroa jacobsoni* occurs on its native host *A. cerana* in Malaysia and Java, while *V. destructor* is found on *A. cerana* on the Asian mainland and on other *A. mellifera* subspecies worldwide (Zhang, 2000). The Asian honeybee, *Apis cerana,* co-evolved with *varroa* and employs innate behavioral mechanisms (e.g., chewing out infested brood) to arrest colony infestations at manageable levels. Additionally, mites cannot develop in *A. cerana* worker brood cells, and are limited to the longer developing drone cells (Spivak, 1996) while drones weakened by parasitism cannot emerge, hence both drone and mite die. In contrast, naïve populations of *Apis mellifera* possessed no innate resistance to *varroa* and suffer alarming population declines on initial exposure.

Varroa mites feed on the haemolymph of larvae, pupae and adult honeybees, during different times of development, and numbers can proliferate to colony-lethal levels if unchecked. Chemical suppression has been commonly employed in America and parts of Europe. While successful in the short term, beekeepers have had to constantly revise their chemical armory in response to chemical resistance developed by mites. After 20 years of often haphazard chemical applications, mites in many countries have developed resistance to much of what was used against them (e.g. pyrethroids such fluvalinate). Italian bees became resistant to this class of chemicals in only 4 years and resistance rapidly spread across Europe. More dangerous chemicals such as the organophosphate coumaphos (Perizin^{TM} or Amitraz^{TM}) are no longer effective in some places (USA, France). Denmark, in contrast to most nations, employed a nationally concerted response when *varroa* was detected. Their approach limited chemical use. Apiaries were encouraged to remove drone cells in the spring (*varroa* prefer drone cells since the longer drone development time allows for better mite survival rates) and apply organic acids (formic and oxalic acid) a couple of times a year. Sixty percent of Danish apiaries detected no *varroa* problems in 2005 with an additional 25% reporting mild infestation of a colony or two (Vejsnæs, 2005). However, with this all said, *varroa* is still a threat to Danish bees. Vejsnæs et al. (Vejsnæs et al., 2010) describe losses of 30% in approximately 12,000 hives over the

winter of 2007-8. Favorable weather allowed *varroa* numbers to increase to lethal levels in many colonies that winter.

1.2.2 Mite resistance in honeybees

Experience has demonstrated that resistant mite populations proliferate under the selection advantage conferred on them by inappropriate chemical applications. An alternative approach to the *varroa* problem has been the establishment of breeding programs selecting for various *varroa*-resistant behaviours (Spivak, 1996; Rinderer et al., 2000). Marla Spivak breeds bees that exhibit hygienic behavior (HYG), a two-step disease resistance process performed by different bees within the colony. Some bees uncap infected calls, while others remove the exposed (dead) brood from the hive (Gramachko and Spavik, 2003). Originally discovered as a response to American foulbrood, the behavior has demonstrated effectiveness against the *varroa* mite (Spivak, 1996). Once considered to be a simple two locus (one controlling capping and the other removal) "on or off" trait, the behavior is now recognized to be influenced by at least seven genes (Lapidge et al., 2002; Wilkes and Oldroyd, 2002). *Varroa* sensitive hygiene (VSH) is a closely related behavior. Bees exhibiting VSH can detect mite infested brood and uncap the cell to remove the live brood, disturbing mite reproduction in the process (Boecking and Drescher, 1991; Rinderer et al., 2000; Harris, 2007). The United States Department of Agriculture (USDA) has been working with *varroa*-resistant strains of *A. mellifera* that adapted in sympatry with *varroa.* European honeybees from the Ukraine were moved to the Primorsky region of Eastern Russia, approximately 100 years ago. These bees adapted to *varroa* in a chemical free environment and were the precursors of the *varroa*-resistant strains released for commercial use in 2000 (Rinderer et al., 2000). Differential gene transcription analyses of *varroa*-sensitive and non-sensitive bees indicated differences in olfactory and neural sensitivity-associated genes (Navajas et al., 2008). Based on these observations, the authors suggest that resistance to *varroa* is mostly behavioral. Identifying the location of relevant loci has proven to be a challenging task since behavior traits are often under the influence of multiple genes, and as previously noted, involves two separate behaviors carried out by two different bees. Recent work from the Behaviour and Genetics of Social Insects Lab, University of Sydney (Oxley et al., 2010) identified six quantitative trace loci (QTL's).

The South African experience is noteworthy since it has been postulated that the lack of chemical intervention and increased hygienic behavior resulted in the observed population rebound. The *varroa* mite (*Varroa destructor*) was detected into South Africa in 1997. Although associated declines in native *A. mellifera capensis* and *A. m. scutellata* populations occurred, no chemical intervention was adopted. After seven years of decline, population numbers began to rebound, and *varroa* resistant proliferated (Fazier et al., 2009). Losses due to *varroa* were recently described as incidental. African bees have demonstrated naturally higher levels of hygienic behavior that other species of western honeybee, demonstrating shorter brood time and greater tendency to swarm. Fries et al. (2006) attempted a controlled version of the above natural 'live and let die' experiment. They demonstrated co-adaption between host bees and mite over a six year period in an isolated bee population of 150 hives. These hives were infested with *varroa* and left untreated. Mite induced winter mortality dropped from 76% in the first year to 13 and 19% in the fifth and sixth years.

Some breeders also recognize the benefits of a more holistic approach to dealing with parasites and disease. Continually medicating against *varroa* for example, can bolster and help propagate disease susceptible strains. Population level tolerance can be enhanced by breeding from the more mite-tolerant colonies, but treatments must be controlled so that colonies with greater and lesser mite resistance can be distinguished. Some regions in the northern hemisphere (e.g. Lleyn peninsula, Wales) are reporting limited mite mediated losses and a concurrent reduction in varroacide use. Commercial beekeeping operations are therefore reducing the use of medication in the production part of their operation, and trying to eliminate treatment altogether in colonies selected for breeding. Research indicates that a balance can develop in closed populations between mite virulence and bee tolerance (possibly due to the viruses they vector) in un-medicated populations (Fries, 2009; Seeley, 2007). Locally adapted bees have demonstrated superior survivorship under no-treatment regimes

1.2.3 Nosema

Microsporidia of the genus *Nosema* are specialized fungi that parasitize many kinds of animals. Three species infect honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) in the U.K.: *Nosema apis, N. ceranae,* and *N. bombis.* These parasites infect gut epithelial cells, weakening individuals and colonies. *Nosema apis* causes dysentery. Several viruses can transfer between individuals via contact and fecal contamination, and

are likely to associate with *Nosema* infection. These include: black queen-cell virus, bee virus Y, and filamentous DNA virus (Ribiére, 2007). *Nosema apis* also causes disjointed wings, increased winter die off rates and slow down of spring build-up of colonies. *Nosema ceranae* was first observed in *A. mellifera* apiaries in Spain in 2006 (Higes et al., 2006). It appears to be the most damaging of the two species (Paxton et al., 2007), having the capacity to cause complete colony failure independent of any other infection (Higes et al., 2009). Dysentery has not been reported as a symptom of *N. ceranae* infections (Fries, 2009). *N. apis and N. ceranae* are currently susceptible to treatment by fumagillin (Higes et al., 2009). Although *N. ceranae* was statistically dismissed as a potential cause of colony collapse disorder (CCD) in the United States (Cox-Foster et al., 2007), it was later reiterated (Paxton, 2010) that the authors recognized that their study was not the best approach to determining the causes of CCD since it was a snap-shot view only, and could not track changes over time. Studies tracking colonies through time (Higes et al., 2009; Martín-Hernanández et al., 2009) have reported mortalities resulting from *N. ceranae* infection. Paxton (2010) also suggests that regional differences to sensitivity to nosema may be due to differences in virulence among different strains of the micosporidian. It seems that the role of *Nosema* in CCD has not yet been clearly elucidated.

1.2.4 Viruses

Viruses are important bee pathogens of great concern and interest to beekeepers and researchers. Over 18 viruses are known to infect bees (Baker and Schroeder, 2008). Most of the common viruses have single strands of positive sense RNA (Table 1.3). Colony life provides a good environment for viral transmission. Viral transmission can occur horizontally and vertically, either passing directly between individuals or from parent to offspring in eggs and sperm (de Miranda and Genersch, 2010). Viruses can maintain intergenerational host/parasite equilibriums through vertical transmission when hives are healthy. Clinical signs of infection may be unobserved under such circumstances. Alternatively, viruses pass horizontally among hive members during periods of stress, passing into haemolymph after mite induced puncture, for example, or being ingested by feeding and grooming in unhealthy hives. *Nosema*-induced dysentery may also aid the viral transmission of BQCV and other viruses. Poor weather conditions can also aid viral replication since hygiene condition may deteriorate within the hive as bees may not be able to leave to defecate.

Viruses have been implicated in the several bee die-off and colony collapse incidences (Bailey, 1964; Cox-Foster, 2007), and are known to associate with other bee parasites. Black queen cell virus (BQCV) has been linked to *Nosema*, and deformed wing virus (DWV) to *Varroa*. Paradoxically, DWV exhibits low virulence in *Apis mellifera* (de Miranda and Genersch, 2010). More virulent bee viruses like chronic bee paralysis virus (CBPV), acute bee paralysis virus (ABPV), Kashmir bee virus (KBV), BQCV, sacbrood bee virus (SBV) may not be suitably vectored by *varroa* since they cause too rapid a demise of its host colony (de Miranda and Genersch, 2010), and don't allow enough time for the mite to reproduce. The 'classic' *varroa*-DWV model recognizes that the negative effects of DWV on bee health are a consequence of complex interactions between the mite, bees, and the transmission pattern and virulence of the virus. Nevertheless, consistent overwinter colony mortality resulting from DWV infection in the absence of mites was recently reported (Highfield et al., 2009).

Table 1.3 Common Bee Virus

Virus		Family	Genus	RNA sense
Acute Paralysis Virus	**APV**	Dicistroviridae	Aparavirus	ssRNA positive
Israeli Acute Paralysis Virus	**IAPV**	Dicistroviridae	Aparavirus	ssRNA positive
Kashmir Bee Virus	**KBV**	Dicistroviridae	Cripavirus	ssRNA positive
Black Queen Cell Virus	**BQCV**	Dicistroviridae	Cripavirus	ssRNA positive
Chronic Paralysis Virus	**CPV**	Unclassified		
Cloudy Wing Virus	**CWV**	Dicistroviridae		
Deformed Wing Virus	**DWV**	Iflaviridae	Iflavirus	ssRNA positive
Sacbrood Virus	**SBV**	Iflaviridae	Iflavirus	ssRNA positive
Kakugo Virus	**KV**	Iflaviridae	Iflavirus	ssRNA positive
Varroa destructor Virus 1	**VDV-1**	Iflaviridae	Iflavirus	ssRNA positive

Source material obtained from the European Commission project report; Virology and the honey bee, 2008 (Ribière et al., 2008; Carter and Genersch, 2008).

It is known that insect can tolerate viral pathogens without showing clinical signs of disease (viral accommodation); an observation that is not clearly understood. Insects probably utilize an anti-viral mechanism termed RNA interference (RNAi). RNAi is a form of post transcriptional gene silencing (PTGS) that was originally detected in flowers. It has since been also observed in insects and animals. A revolution in RNAi based gene silencing technology has occurred over the last ten years. The USDA is currently running clinical trials on a new RNAi based treatment for the honey bee virus Israeli Acute paralysis Virus (IAPV) (Maori et al., 2009). Israeli bee paralysis virus was identified as a potential marker for colony collapse disorder (CCD) (Cox-Foster, 2007) and was a good candidate for RNAi. Beeologics, a biotechnology company from Israel, have taken

advantage of the RNAi mechanism to develop anti-viral treatments for bees. They claim to have developed a treatment that offers potent protection from the following bee viruses: Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), black queen cell virus (BQCV) and deformed wing virus (DWV).

The most common bee viruses are RNA-based (Table 1.3). Polymerase Chain Reaction (PCR) technology allows for detection and quantification of viral activity in bees. Bees can be screened for specific viral infection by applying reverse transcription of viral-specific mRNA, followed by amplification and visualization of the resulting cDNA. Baker and Schroeder (2008) demonstrated that the RNA dependent RNA polymerase (RdRp) gene can reliably distinguish between viruses within the Picornavirales, an order that includes many of the common bee viruses. They also suggest that DWV, VDV-1 and KV from the genus *Iflavirus,* are variants of the same virus and that care should be given in using species-specific' primer sets within that genus. Real-time quantitative PCR technology allows viral loads to be quantified. This procedure detects material that is only produced when the virus is actively replicating, indicating that an active infection is occurring. The detection and quantification of replicated negative strand RNA would suggest a true infection is occurring as opposed to passive viral transmission (de Miranda and Generch, 2010; Gisder et al., 2009)

1.2.5 Pesticide Threats

Due to the nature of farming in Wales, local honeybees are not likely to be greatly affected by pesticides. Nevertheless, bees are susceptible to pesticides and recent work on the honeybee genome has shown that relative to other insects, they have fewer genes coding for detoxifying enzymes (Claudianos et al., 2006). Recent worldwide developments have also highlighted concern regarding the increasing use of neonicotinoids, a specific class of pesticides. Neonicotinoid treated seeds offer systemic protection to the developing plant, and are now commonly applied to many commercially important crops (e.g. corn, oil seed rape, sunflowers) on an industrial scale. All parts of the plant (including pollen and nectar) are pesticide laden. A coalition of beekeepers and environmental groups recently sued the United States Environmental Protection Agency (EPA) for approving the registration of Clothianidin and Thiamethoxam, claiming that these neonicotinoids cause severe damage to bees and are the primary cause of colony collapse disorder (CCD). Recent scientific publications have provided evidence supporting such claims. A high profile paper by

Schneider et al. (2012) described how neonicotinoids induced CCD like symptoms (including a vacated and empty hive) in experimentally exposed colonies. Neonicotinoids are acetylcholine receptor agonists that bind irreversibly causing hyper-stimulation of the nervous system. These effects adversely affect the brain function and thought that foraging field bees become disoriented and fail to return to the hive.

Another recent high impact paper claimed that field concentrations of neonicotinoid pesticides can detrimentally affect queen health and the development of bumble bee colonies under laboratory conditions (Whitehorn et al., 2012). There is a growing body of evidence implicating pesticide use with pollinator loss, and on the April 29th 2013, the European Union responded by voting to enforce a 2 year ban on the use of three type of neonicotinoids on flowering plants, though eight (including the UK) of 27 member states voted against the ban, and four abstained. Those doubting the ban claimed that scientific evidence is currently inconclusive and that a complete embargo is unwarranted.

Opinions are similarly divided among the beekeeping community. Some commercial operators have observed no adverse effect on their bees while foraging on neonicotinoid treated crops, and claim that lack of *varroa* mite control and poor forage quality due to shifts in climate patterns and agricultural practices are more impactful causes of colony loss. Randy Oliver, a scientifically trained commercial beekeeper from California (scientificbeekeeping.com), recently wrote a critique of the Schneider et al. paper (Oliver, 2012). He questioned both the methodology used (which involved very high neonicotinoid loads presented to the experiment colonies) and their interpretation of results. He suggests that the observed colony losses could have resulted from ineffective mite control, rather than from pesticide poisoning. He presented these concerns in writing to the authors but has yet to receive a response. A contrasting opinion is presented by another group of American commercial operators, some of whom lost up to 70% of their colonies this winter. The journalist Dan Rather (2013) reported on the resulting shortage of bees for almond pollination in California this spring. Neonicotinoid pesticides were considered by many to be a major contributing factor affecting declining bee health.

Chemical treatment has also been the prescribed response by many to *varroa* mite infestation. *Varroa* frequently developed resistance, necessitating the use of novel chemical treatments. Some of these chemicals could accumulate in the hive with time and

have unwanted effects at the higher concentrations. New treatments are developed in response. The arms race continues in large scale bee operations.

1.3 *Bee Translocations*

The western honeybee evolved across a wide range of ecological and climatic conditions (Moritz et al., 2005). Separate races or sub-species became regionally adapted, developing regionally specific phenotypic and behavioral characteristics suited to particular environments and conditions. Technological developments allowed bees to be distributed away from their endemic ranges. Moritz et al. (2005) describe three kinds of human mediated distributions: spread of *A. mellifera* within the ranges of other *A. mellifera* sub-species (Europe, Western Asia and Africa); distribution of *A. mellifera* sub-species in regions where other species of the genera *Apis* were found (Asia); and translocations into areas not endemic to honeybees (Americas and Australia).

Foreign ecotypes (sub-species) exhibiting 'superior' traits have been introduced into the UK over the years in an effort to enhance beekeeping productivity. Queens under natural conditions mate on the wing some distance from the nest. They can therefore come into contact with drones distant colonies. Consequently, both the managed and wild British honeybees are probably of mixed genetic backgrounds. The plight and condition of native bee populations is presently unclear. The introduction of *Varroa destructor* was undoubtedly detrimental. At worst, the combined effects of disease and the introgression of genes from introduced bees may have resulted in the extirpation of the native bee. Nevertheless, bee colonies are cryptic and hard to locate, and locally adapted wild bees that are in 'balance' with the parasite, may exist in some remoter parts (Jensen et al., 2005; Villa et al., 2008). A number of regional bee breeding cooperatives are attempting to identify and conserve these bees.

1.3.1 Translocation within the endemic A. mellifera *range*

Beekeepers have moved bees around the world in an effort to enhance desired beekeeping traits. Since bee reproduction is difficult to control, introgression of genes from introduced into native bee population can easily occur, resulting in the breakdown of locally adapted gene complexes. In addition, areas can be flooded with managed queens of limited genetic variation. Lack of genetic variation would weaken population level response to environmental threats, and result in poorly mated queens (mated with few individuals or

closely related individuals). Hives with poorly mated queens have less resistance to pathogenic infection (Baer and Schmid-Hemple, 1999; Hughes and Boomsma, 2004; Seeley and Tarpy, 2007). Although the integrity of regionally co-adapted gene complexes have been challenged by bee translocation, research suggests that *autochthonous* sub-species can still be found in parts of Europe (De la Rua et al., 2001, 2002, 2003; Jensen et al., 2005; Strange et al., 2007). In addition, notable efforts have been made to preserve native strains. The Danish government implemented conservation measures to protect the endemic "black" honeybee on the island of Læsö. Introgression of non-native genetic material has occurred as a result of illegal importation of other *A. mellifera* sub-species (Jenson et al., 2005).

Two sub-species of *A. mellifera* are endemic to South Africa, *A. m. capensis,* and *A. m. scutellata*. Translocation of *A. m. capensis* into the native range of *A. m. scutellata* for commercial beekeeping purposes resulted in rapid disappearance of the *A. m. scutellata* colonies (Neumann and Hepburn, 2002). *Apis mellifera capensis* workers parasitized *A.m. scutellata* hives, superseding native queens, and took over colonies by becoming layers (Neumann and Hepburn, 2002; Moritz et al., 2005). Commercial beekeepers suffered great losses, but native wild *A.m.scutellata* have to date been relatively unaffected

1.3.2 Translocations of A. mellifera *into the native range of other* Apis

Apis mellifera has become popular with Asian beekeepers, causing considerable decline in use of the native *A. cerana* (Moritz et al., 2005). Hybridization can occur in both directions between the species (Moritz et al., 2005). The negative consequences of hybridization have been well documented (Allendorf et al., 2001). Hybridization between these two species results in reduced fitness since queens of either species will be poorly mated resulting in the waste of reproductive resources (Moritz et al., 2005). The hybrid juveniles are inviable; hence locally adapted *A. cerana* gene complexes stay intact. The transfer of the parasitic mite *Varroa desctructor* from its native host *A. cerana,* into naïve *A. mellifera* populations, initiated the most devastating plague of the western honeybee (Moritz et al., 2005). Its spread has highlighted in a dramatic way the unintended consequence and dangers of ill-informed translocations. *Nosema ceranae* is also thought to have recently transferred from *A. cerana* to *A. mellifera* and is expressing increased virulence in its new host (Fries, 2009). Significant colony losses recently reported in Spain were attributed to *N. ceranae* parasitism.

1.3.3 Translocation of A. mellifera *into regions with no indigenous* Apis

There are no honeybees endemic to the Americas and Australia. The first American honeybees were probably British black bees (*Apis mellifera mellifera*) which landed in Jamestown in 1622 (Delaney et al., 2009). Feral bees moved west across the continent to the eastern slopes of the Rockies. No bees made it across the mountains. The first honeybees to make west of the Rockies arrived in California by boat in the 1850's. Most honeybees (*A. m. carnica* and *A.m. ligustica*) were imported between 1859 and 1922. Importation of bees into the US was outlawed in 1922 in response to the 'Isle of Wight' disease that had decimated British bee stocks. The ruling limited genetic variation in available breeding stocks. It is thought that the progeny of all the commercial hives in the US were bred from only 500 breeder queens (Delaney et al., 2009). Low levels of genetic diversity correlate with reduced disease resistance, colony strength and overall colony fitness in bees and other social insects (Tarpy, 2003). In addition, genetically similar colonies are less buffered against disease transmission between colonies, and are at greater risk of high colony losses.

1.4 *Colony Life*

The type of advanced colonial structuring that is observed in honeybees is termed eusocial. It is characterized by cooperation between individuals in brood care and nest construction, overlapping generations, and reproductive division of labor (Wilson and Holldobler, 2005). A normally functioning honeybee colony may have 60,000 or more individuals, consisting mostly of female workers that perform within and outside hive tasks such as brood care (nursing), nest defense and foraging. Workers also tend to the queen, the prolific egg-layer and mother of the colony, whose task it is to encourage colony growth and ultimately reproduction through swarming. Each colony will also contain males (drones) at certain periods of the year. Far fewer in number than workers, they are specifically adapted to detect, catch, and mate with queens during their nuptial flight(s). Drones mate only once. Virgin queens undertake one to three mating flights within the first few weeks of life, mating with multiple males (drones), and storing the sperm for lifetime use and storage. The mean paternity frequency (i.e. actual number of matings) for *A. mellifera* is around 13 (Cournet et al., 1986; Estoup et al., 1994). Seeley and Tarpy (2007) demonstrated that colonies with higher levels of genetic variation (i.e. greater number of patrilines) were less affected by American Foulbrood inoculation than colonies formed by single mated queens. Baer and Schmid-Hempel (1999) reported similar results with bumblebees (*Bombus*

terrestris L.) with greater genetic variation correlating with reduced pathogen loads and better reproductive success (see also Hughes and Boomsma 2004; Palmer and Oldroyd, 2003).

Extreme polyandry (>2 matings per queen) is relatively rare among the highly eusocial insects (Tarpy and Page, 2000). It occurs in a few wasps, ants and bee genera, and has been the topic of much debate, since it is not intuitively obvious what selective advantage(s) is confers. Polyandry reduces the degree of relatedness among colony individuals and exposes the queen to environmental (predatory and pathogenic) threats (Tarpy and Page, 2001). In addition, within hive genetic heterogeneity has been correlated with greater thermoregulation efficiency. Controlled experiments demonstrated that genetically diverse colonies (greater number of patrilines) displayed greater thermal stability in response to environmental change that genetically poor ones (Jones et al., 2004).

1.5 *Complementary sex determination gene* csd

Sexual development in Hymenoptera is directed by a specific genomic region (Sex Determination Locus; SDL) found on chromosome 3. Within this locus resides the complementary sex determination gene (*csd*), whose protein product initiates the development of males (usually haploid) in the default state. However, when the protein product of two functionally distinct alleles combine (i.e. in diploids), another gene within the SDL (*fem*) is switched and the process of feminization is triggered. Feminization occurs only when *csd* alleles differ in diploids; homozygotes develop into sexually in-viable diploid drones and are 'cannibalized' at an early developmental stage by workers. Strong frequency dependent selection and heterozygote advantage promote high gene variance at the locus. High levels of polymorphism are observed due to these forces (balancing selection) since alleles tend to persist in evolutionary terms.

The population dynamics of the *csd* is of relevance to the bee breeder since colonies with low brood viabilities due to unacceptably high levels of diploid drone production will be less productive. Queens mate multiple times, and the probability that she will mate with a drone carrying an identical allele to one of the two she carries is $2/k$, where k = the number of alleles in the population (assuming each is present in equal proportions). From this relationship Page and Marks (1982) deduced that the brood viability (V) of a queen that mates *n* times, with *y* of those drones carrying alleles that matched one of her own, is,

$$V = 1 - \frac{y}{2n}$$

This relation assumes that each drone has an equal probability of mating and provides an equal amount of sperm. In addition, the expected brood viability in a population closed to the influence of migration will be

$$= 1 - \frac{1}{k}$$

The expected mean brood viability is therefore higher in population carrying higher numbers of distinct alleles since the probability of identical alleles matching in zygotes is reduced. In addition, the mean population mating success (mean number of drones each queen mates with) affects the variance in population level brood viability, but not the mean itself (Cook and Crozier, 1995), with lower mating success resulting in greater variance in brood viability. Number and frequency of distinct alleles (*k*) are important population level criteria affecting diploid drone production. In general the industry considers brood viabilities of less than 85% as unacceptable (Page and Marks, 1982). Beekeepers trying to direct adaptive change by selecting a limited number of breeders each year will limit the transfer of gene variation across generations, by they must concurrently maintain the number and frequency of sex alleles to maintain an acceptable levels of brood viability in the long term.

The molecular mechanisms of single locus sex determination are not completely understood. It is not yet known for example, how one *csd* allele differs from another. A hypervariable region (HVR) located in region 3 of the gene most likely holds the key to unravelling this riddle (Cho et al., 2006). The HVR can be described as a pseudo-microsatellite since it is comprised of short repetitive sequences, bounded by an arginine and serine rich region on one side, and a proline rich region on the other. These more conserved bordering regions were targeted by PCR in this study to investigate fragment length variation within the HVR. One hypothesis suggests that the number of HVR sequence repeats characterize *csd* allele function, and that differing numbers of repeats at this coding region result in protein products of correspondingly differing lengths and possibly function (Cho et al., 2006).

1.6 Bee Breeding

1.6.1 Hybrid Breeding

Selective breeding methods have been adopted for centuries to improve agricultural strains of plants and animals. More recently, the genetic influences underlying the beneficial effects of heterosis (hybrid vigour) have become better understood and recognized by plant and animal breeders (Shull, 1948). Beekeepers have also realized the potential benefits of out-crossing and the method has been successfully applied to improve stock vigor (Cale and Gowen, 1956). However, since hybrid breeding requires the long term and costly maintenance of pure inbreed lines, such efforts usually required the resources of large commercial operations or research facilities. The Starline and Midnite bees were once popular commercial four line hybrids produced by Dadant and Sons, Inc. (United States); each was continually improved by the addition of new hybrid lines. Advancements in Instrumental Insemination (II) methodologies (Laidlaw, 1944; Mackensen, 1947) allowed breeders to maintain and cross genetically isolated lines through artificial mating. The technique continues to be used to control mating. It does require some specialized equipment and training; hence it is mostly used by professional breeders and research establishments.

1.6.2 Line Breeding

A more commonly used approach is line breeding. Line breeding has been used since the middle of the nineteenth century by European and American breeders. Most famously in the UK, brother Adam of Buckfast Abbey developed the Buckfast line through many years of cross-breeding different lines of geographical sub-species. He did this using open mating partly in response to colony losses from the Isle of Wight disease during the early part of the 20th century. Contemporary breeders mostly use line breeding to strengthen honeybee stocks by encouraging the propagation of beneficial traits within the gene-pool.

A model line breeding program (The Russian Bee Breeding Program) was established by the United States Department of Agriculture (USDA) in the 1990's. The program was transferred, with federal support, to the commercial sector and is currently maintained by the Russian Honeybee Breeders Association, Inc. Seventeen lines, divided into three separate blocks A, B, and C, are currently maintained. Blocks are comprised of a number of independent beekeepers, each maintaining no more than two lines. An intricate breeding design (Fig1.1) has ensured that inbreeding effects are minimal, both within the program, and within the stock provided for commercial sale.

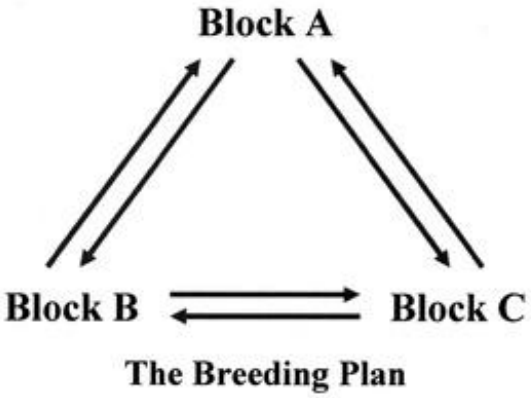


Figure 1.1. Each year, members will select the best looking colonies from each of their lines as breeders. Their daughter colonies will be mated by drones sourced by queens donated from the other two blocks. For example, a beekeeper maintaining lines in block A will mate his virgin queens with drones produced by queens provided by all the members of block B and C. A large number of daughter colonies are raised, and these are also distributed among the other blocks for monitoring different environmental conditions. In order to limit detrimental inbreeding effects, queens are made available for commercial sale from each block only every third year.

Table 1.4 Bee breeding programs

Breeding Programs	
Conservation	
Conserving the Dark Bee in Europe	http://www.gbbg.net/
Conserving the European Dark Bee, Germany	http://www.apis-mellifera-mellifera.de/
Saving the Dark Bee in Switzerland	http://www.mellifera.ch/
Bee improvement in Cornwall	http://www.westcornwallbka.org.uk/member/
Bee improvement and Breeders Association	http://www.bibba.com/
Disease Resistance Programs	
Russian honey bee (Ontario, Canada)	https://www.uoguelph.ca/ses/users/eguzman
Minnesota Hygienics Program	http://www.glenn-apiaries.com/hygienic_italian_
Russian Honeybee Project (US Dep. Agri.)	http://www.ars.usda.gov/Services/docs.
Varroa-tolerance New Zealand	http://www.biosecurity.govt.nz/publications/biosecurity-magazine

There are numerous programs adopting similar approaches worldwide (Table 1.4). Some programs prioritize the enhancement of autochthonous phenotypes, believing that locally adapted bees are better suited to regionally specific environments. For example, the

widely introduced Italian bee (*A. m. ligustica*) may not be well suited to forage and overwinter in temperate northern European climates

1.6.3 Closed population breeding and selection

Closed population breeding is the process of selecting for specific required traits from a closed population of bees. Closed populations are genetically isolated, and can be thought of as a single line. Populations can be large or small, and more or less closed (Kulinčević, 1986), and various selection strategies (e.g. mass, random, within-family) can be employed to select breeders (Figure 1.2).

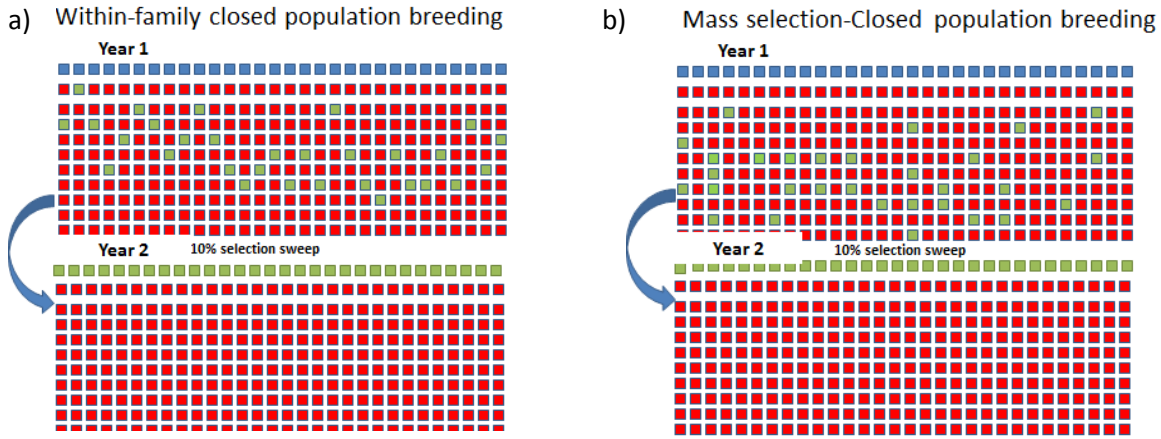


Figure 1. 2. Ten daughter colonies (red) are raised from each selected breeder (blue) in year 1. The best performing daughter colony (green) from each breeder line (within family) is selected as a breeder (fig 1.2a). In contrast, year 2 breeders are selected without concern for family line in mass selection (fig 1.2b). Expressed character traits and performance are the most significant considerations in this case. Breeders can also be selected at random.

1.6.4 The West Wales Bee Breeding Program

The West Wales Bee Breeding Program (WWBBP) was set up as a collaborative enterprise between Bangor University and Tropical Forest Products; a commercial honey producing and bee related business based in north Dyfed. Its formation was motivated in part by the gradual decline in bee health observed over recent years. Colonies continue to succumb to the ravages of *varroa* and the bee-related viruses they carry. In addition, the region has suffered a series of particularly poor summers; a climactic trend that has forced beekeepers to use increasing amounts of supplemental feed to avoid losing colonies to starvation. There is also concern that queens might be struggling to mate successfully and prematurely failing due to this persistently poor weather. Beekeepers in other parts of the northern

hemisphere have consistently stated prematurely failing queens as a main reason for overwintering losses (vanEngelsdrop et al., 2008, 2011, 2012). Honeybee queens mate multiply on the wing, usually some distance from the nest, and will do so more successfully during good weather. Young sufficiently mated queens tend to develop into healthier, more vigorous and longer lived individuals than less successfully mated queens. Queen mating success has been shown to influences the long term development and performance of colonies (Richard et al., 2007; Tarpy et al., 2012).

Commercial beekeeping has become an increasingly risky proposition due to declining bee-health. In response, some beekeepers have strived for sustainability by breeding from locally proven productive stocks, rather than relying on imports to replace losses. Strange et al., (2007) showed how bees adapted to regionally specific nectar flows, are ill-prepared when moved to areas where peak nectar flows occurred at different times. Much of the managed bee stock is now of mixed genetic heritage, and may therefore not be well suited to all regions. Bees that evolved in northern climates for example, delay brood expansion until late spring. Hybrids tend to expand earlier in the year and are more susceptible to starvation if weather conditions turn unexpectedly cold. Hybrid queens cannot adjust their egg-laying in response to weather and their colonies may not be able to survive without supplemental feeding (Le Conte and Navajas, 2008). Honeybees have evolved in a broad range of environments, and breeders hope to take advantage of this innate diversity (plasticity and genetic) to breed for local adaptation (Le Conte and Navajas, 2008).

The challenge for the WWBBP was to design a purposeful breeding program that could be integrated into the management framework of an existing small commercial operation. Within this context, the aim was to start developing a breeding protocol that could maintain a self-sustaining and productive population over the long term. There are no fixed or defined end points or goals; only a process that enhances the resilience of bees to be responsive to ever-shifting climate and disease challenges. It is an applied long term project hoping to improve the commercial quality and regional specificity of a managed honeybee stock.

The breeding program started in the spring of 2011. Tropical Forest bees suffered high mortality over the 2010/11 winter and priority was given that summer to re-building colony numbers. An estimated 43% of the Welsh colonies succumbed, with *varroa* mite

infestation deemed to be the major contributing factor. Potential breeders were selected from overwintered survivors dispersed in apiaries up and down the Dyfi valley (mid-Wales). The situation offered a breeding opportunity since a large number of new colonies (n = 118) were needed to recoup losses. This was a rather unusual situation, since this many replacement colonies are not normally required. The business accommodates at most two hundred colonies in mid-Wales and experiences roughly 30% loss (60 colonies) each year.

Beekeepers use various techniques to replace losses. Unfortunately, each method requires dividing (splitting-glossary) the resources of strong colonies, regressing their progress and future production potential in the process. The 'old' reduced colony usually retains the original queen. All 'new' colonies require new queens which can be acquired through a number of different ways. Queenless splits can be left unattended near the original hive with eggs and/or brood of appropriate age so that the bees can raise new queens (walk-away split). Alternatively, the splits can be relocated and provided with an already mated laying queen, or a ripe queen cell from which a virgin will imminently emerge. None of these approaches provide immediate fixes since each new colony can take a season, if it survives, to mature into production size in the UK. These are familiar beekeeping practices that have been used by beekeepers managing sustainable programs to replace expected seasonal losses. But increasingly severe losses result in more strong production hives having to be sacrificed to make up colony numbers. Managing bees for honey production has become increasingly difficult in the UK and is in danger of becoming commercially unsustainable.

Having timely access to well-developed and genetically appropriate queens can provide commercial operators with greater management flexibly. Replacement queens of reliable stock are not readily or cheaply available in the UK. A limited number of sources do exist, but relying on availability, sometimes weather dependent, from second party producers complicates program planning. Ripe queens are too sensitive to temperature shifts and movement to be easily shipped via mail and must usually be picked up in person at a pre-arranged time for example. Due to ease and convenience therefore, beekeepers frequently use walk-away splits to replace losses. Reproductive swarm cells are thought to produce the best queens and can be removed from choice colonies as they prepare to swarm, but this approach is not normally practiced as beekeepers are keen to suppress the swarming

impulse. Otherwise beekeepers have little control over the replacement process as queens raised in emergency situations (as in walk-away split), particularly in dearth conditions will be of inferior quality due to lack of nutrition during development. Nevertheless, this form of hive management is commonly practiced in the UK (Carreck and Neumann, 2010). As an alternative approach, the establishment of an independent in-house queen rearing programs can offer small scale commercial operations economic benefits through reducing costs and increased flexibility. Periodical rounds of grafting and rearing could provide, with fairly minimal effort, a steady supply of replacement queens. These benefits could help beekeepers better manage recovery from loss, and maintain a higher mean number of production size colonies. In addition, failing queens could be replaced with queens raised from locally proven productive stock. Successful programs have demonstrated that incremental progress towards a healthier more productive bee population is possible by continually breeding from only the best performing colonies. But the process is continual and will take several generations since there are no defining end points on goals.

Historically, the focus in apiculture has been directed toward selecting appropriate queens. Drones are often neglected as targets of selection. This is due in part to the limited control of drone mating activity, and to the fact that most traditional selection characteristics are expressed by the queen. Queens clearly have great influence over overall colony characteristics, but more attention could be directed toward drone selection. Increased rates of queen failure (possibly due to poor mating success) have been reported in Wales over recent years.

There could be differential rates of mating success among drones of different genetic backgrounds, and the potential influences of parasitism and disease need to be elucidated. In addition, climate cycles over recent years dictate that bees in Wales need to successfully mate during short periods of good weather. Monitoring the cool weather flying behavior of queens and drones during these times might help us understand the influence of weather on the mating success of current bee stocks.

1.7 Aims of this thesis

Wales commonly experiences periods of low temperatures and high precipitation, but has recently suffered a series of particularly wet and cold summers. Beekeepers in the region have coincidentally noted increased rates of premature queen failure and it is possible that

these suboptimal breeding conditions may have restricted mating. I assess how well queens from this managed population mated under local conditions during the summer of 2010, and recorded queen flight response to environmental challenge during this critical developmental period (Chapter 2). Chapters 3 and 4 examine the phenotypic and genetic consequences of selection performed in 2011 and 2012. In Chapter 5 I describe the development and use of Monte Carlo simulation models to investigate how various selection parameters (e.g., number of breeder queens, mating success, and population size) can influence genetic change (changes in allele frequencies) in a small honeybee population within a contemporary time frame. Model predictions were compared to real population data when available (two generations of selection), and simulated genetic change for 5 generations of selection in total. Comparisons were made using two different models; one designed to accommodate neutral markers, and the other with a locus under selection (*csd*). The final experimental Chapter (Chapter 6) investigates sex allele (*csd*) variation in the source population. Although this locus is of special concern to bee breeders, its mechanism of function is not yet fully understood. I briefly discuss this topic in relation to relevant data acquired from the test population.

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Chapter 2

The mating frequency and flight behaviour of honeybee queens on the edge of their natural distribution

This chapter is formatted for journal publication

The mating frequency and flight behaviour of honeybee queens on the edge of their natural distribution

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Wales lies on the north-western margin of the natural range of the western honeybee (*Apis mellifera*). The region commonly experiences periods of low temperatures and high precipitation due to profound northern maritime influences, but has recently suffered a series of particularly wet and cold summers. Beekeepers in the region have coincidentally noted increased rates of premature queen failure and it is possible that these suboptimal breeding conditions may have restricted mating. We assessed how well queens from a managed population mated under local conditions, and recorded queen flight response to environmental challenge during this critical developmental period. The flight activity of thirty experimental queens, as well as relative environmental variables, was monitored during the 2010 breeding season. Mating success was determined by sampling experimental queen brood and using seven microsatellite markers to reconstruct the number of sib-ships per colony sample. Weather conditions were again uncharacteristically bad during the summer of 2010. Only twenty of the thirty queens managed to establish mature colonies. Mating frequencies ranged from 4 to 10 drones per queen and were below the accepted species mean of 13. We discuss whether queens adjust their flight behavior in accordance with environmental cues and consider the effects on poor mating on ultimate colony health. This work highlights a possible detrimental effect of long term shifts in climate patterns on the activity of managed pollinators.

Introduction

The new century heralded increased stress for honeybees (*Apis mellifera*) in the northern hemisphere. Drastic declines in colony numbers have since been observed across Europe and North America [1]. Beekeepers and researchers have struggled to find sustainable solutions due to the multifactorial nature of the problem. Synergy between contributing factors has further complicated diagnosis and treatment [2, 3]. Parasites (particularly *varroa* mites), viral, fungal and bacterial pathogens, lack of genetic diversity, pesticides,

and starvation, all detrimentally affect the health of honeybee colonies. There is also increased concern about the longevity of commercially reared queens. In a survey of 305 beekeeping operations in the US [4], inferior queen quality was given as the main reason for colony loss during the 2007-2008 winter. Similar reports were published in 2010 and 2011 [5, 6]. Increased rates of premature queen failures have also been observed in managed colonies in parts of Wales (D. Wainwright, pers. comm.; Meirionnydd Beekeepers Association, pers. comm., 2012). The UK's Food and Environment Research Agency suspect disease as a possible cause, but poor mating due to prolonged periods of inclement weather could also be responsible. Wales is located on the north-western fringe of the natural distribution of the honeybee and its climate is influenced by both North Atlantic weather fronts and the elevated topography of much of the country. The region has also recently suffered a series of exceptionally wet and cool summers, a trend that in part reflects its location and elevation, but may also be due to permanent shifts in global climate patterns.

Unacceptably high rates of queen failure are costly for small scale commercial operations. Colony failure results in loss of production potential and may require an additional expenditure of time and money to remedy. Queen vitality is of critical importance to commercial beekeepers since colony health and productivity are closely related to the condition of the queen. European bee-breeders have been selecting for commercially desirable traits (productivity, colony size, temperament,) as indicators of queen vitality since the end of the 19th century [7]. Popular subspecies (such as *A. m. carnica* and *A. m. liguistica*) have been moved extensively outside their native ranges in the process, and have hybridized with bees native to other regions, thus potentially introducing traits not adapted for the unpredictable weather conditions in more northern areas. The genetic background of our experimental bees is unknown but is derived from a commercial stock that has been used for commercial bee-keeping in Wales for many years. Jensen et al. [8] found evidence of genetic introgression of *A. m. liguistica* and *A. m. carnica* microsatellite alleles into putatively pure *A. m. mellifera* populations in Britain, indicating that British bees are commonly of mixed backgrounds. Anecdotal morphological and behavioural evidence also suggest that these bees are of mixed genetic heritage.

Independent of genetics, queen health and performance is also influenced by environmental variables experienced during development [9]. Queens must pass through

three early developmental phases: (pre-emergence, pre-mating, and post-mating) [10] on the path to egg laying and maturity. Each is responsive to specific combinations of environmental variables. For example, larvae develop into healthier bees if they are nourished by pollen from diverse sources [11]. Abundant nectar flows are particularly important for all aspect of queen health [12] and high nurse bee densities are needed for optimal rearing. Breeders can supplement larval needs, and have influence over rearing during this period.

Western honeybees (*A. mellifera sp.*) are cavity nesters that can precisely buffer their nest environment against external influences such as climate [13]. While colony life offers shelter from environmental perturbations, individual bees are susceptible to inclement conditions outside the nest, and none more so than virgin queens during mating flights. Virgin queens emerge into a stable, protective environment, but must subsequently enter a treacherous 14-day developmental phase during which they are most receptive to mate [14, 15]. Queens mate on the wing at drone congregation areas (DCA's) commonly one km or so away from the colony. Here they meet and mate with drones that fly in from surrounding colonies. The behaviour of bees during the period surrounding this critical event has been extensively studied. It is known that queens will leave for their first mating flight when 5-6 days old, and fly an average of 2-5 times [16]. There are two accepted types of queen flights; short flights lasting 1-10 minutes for local orientation, and longer mating flights, lasting up to 30 minutes. Queen honeybees can mate within the first post-emergent week [17] and will start laying on average six days after initiating mating flights [18]. These studies show that queens can start laying eggs within two weeks of emergence. Similarly, an extensive review of 19 years of data from the Breeding Evaluation Center in Germany [10] determined a mean pre-oviposition (from emergence to egg-laying) period of approximately 16 days (range 6-34) from over 3500 *A. m. carnica* colonies. Virgins need extended periods of dry sunny weather in order to mate well, and are vulnerable during this time since they need to leave the nest for prolonged periods. Beekeepers have limited control over their behaviour and fate during this time. Queens mated within 14 days of emergence exhibit superior physiological development and ultimately enhance colony fitness [10], while older virgins tend to mate with fewer drones and have fewer sperm stored in their spermathacae [14, 15]. Increased rates of cell death were reported in the ovaries of queens that had delayed mating [19, 20]. This is a critical

developmental period since queens need high insemination success and mating numbers to acquire optimal mated 'health' [21].

Polyandry in Apis

There is a wide range in the degree of polyandry reported in honeybees (*Apis)* extending from a mean of 8 in *A. florea*, to 27 in *A. dorsata* [22]. A mating frequency of 12-13 is commonly reported for the western honeybee (*Apis mellifera*) [23], but there is evidence of variation among sub-species [24]. A review of studies that used molecular techniques for assessing paternity [25] reported mean values of 15.2 and 13.8 for *A. m. mellifera* and *A. m. carnica*, respectively (two sub-species considered well adapted to, and commonly managed in, northern European apiaries).

Genetic variance and the benefits of multiple mating

Mated queens fertilize their eggs with sperm acquired during nuptial flights. Insemination quantity (and possibly mating number), also has a profound effect on the physiological development of post-mated queens [26]. Virgin, single-mated, and multi-mated queens have different queen pheromone and brain gene expression profiles that strongly influence colony behavior and fitness [26]. As the number of paternal contributors (i.e. number of matings) increases, the number of full-sister sub-families within the colony similarly increases. Colonies with multiply mated queens have been shown to have greater adaptive fitness than colonies with lower levels of genetic diversity through increased foraging efficiency in different environments, greater resistance to parasitic challenge [27-29], enhanced ability to buffer against environmental fluctuations [13], and higher brood viability due to reduced incidence of diploid male production [30-32]. Diploid drones result when identical complementary sex determination (*csd*) alleles match in diploids at the sex determination locus [33]. This occurs less frequently in population with lots of sex allele variation due to chance alone, but mating frequency is important since the variance in brood viability around the population mean is inversely related. The variance in brood viability is therefore a function of both the number of alleles in the population and the mean number of mates each queen pairs with [30]. Most queens will have brood viabilities close to the population mean at higher mating frequencies.

Weather and drone density/abundance

It is known that climate can influence mating frequency [34] and queens that as a result receive insufficient semen will eventually lay only drones and the colony dies. Queens respond to environmental cues and remain confined to the hive during extended periods of cold, wet, and windy days. Lensky and Demter [17] report reduced mating activity occurring below 20 ºC and when the wind speed is above 4-5 ms^{-1}. It is also commonly stated that drone abundance is an important criteria affecting mating frequency, although Neumann et al. [34] found no correlation between mating success and number of drone producing colonies at the mating yard.

We studied the influence of climate on the flight behaviour of locally raised and managed queens, and assessed whether average mating success was constrained by environmental effects. Specifically, we recorded flights times and compared the duration of putative non-mating and confirmed mating flights; testing in so doing the hypothesis that mating flights would be of significantly longer duration. We also assessed the influence of climate on flight duration, and tested the hypothesis that flight duration (hence mating opportunity) would be reduced when conditions were inclement. We used genetic methodologies to determine the mating success of test queens, and tested for correlations between the numbers of paired matings observed, and the weather conditions recorded when mating flights occurred. Multiple mating flight were expected in some cases, and we tested the hypothesis these queens will have greater mating success than queens who flew less frequently.

Our results suggest that the cool and wet conditions experienced during the experimental breeding period (in summer 2010) adversely affected mating success, which was on average well below the accepted species mean. These results possibly arise from both the region's marginal location as well as recent climate shifts. The longer-term consequences of this remain to be seen.

Result

Two batches of fifteen virgin queens were raised using a standard Cloake board queen rearing method [35] during July and August 2010. The first batch of day 10 queen cells was introduced to 6 frame nuclei on the 6th of July, and the first queen was observed in an entrance cage six days later. The first confirmed mating (visual observation of mating

sign) occurred on the 21st of July, approximately two weeks post emergence from cell. An additional five queens were mated two days later (Fig. 1).

The second batch of cells was introduced to nuclei on the 21st of July, and the first queen was observed in a caged entrance nine days later (July 30th). The first confirmed mating flight by a queen from this batch occurred on the 7th of August, approximately 16 days post emergence. Additional mating flights were confirmed on the, 8th, 10th and 14th of August (n = 8, 5, and 3 respectively; Fig. 1).

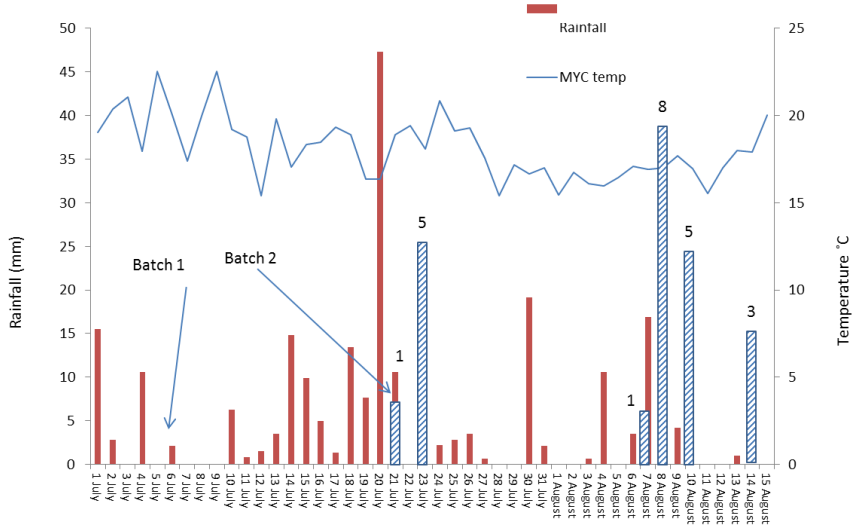

Figure 1. Climate and mating flight behavior at the mating apiary during summer 2010. Two batches (1 and 2) of ripe queen cells were introduced about two weeks apart into pre-prepared nucleus hives. Solid bars and line indicate the total daily rainfall (mm) and the mean peak flying period (afternoon) temperatures. Striped bars and accompanying numbers indicate confirmed mating flights (visual confirmation of mating sign).

A total of 251 queen flights were observed (Fig. 2). Most flights were of short duration (<5min), and not related to mating events (Fig. 2). Due to a highly skewed distribution, the flight duration data were log transformed to conform with the assumption of normality ($P < 0.001$, Kolmogrov-Smirnov test statistics = 0.13 post transformation). There was a significant correlation between flight duration and apiary (MYC) temperature ($P = 0.002$,

Pearson's Correlation = 0.199, N = 243) and relative humidity ($P < 0.001$ Pearson's Correlation = -0.432, N = 243). No correlation was observed between flight duration and windspeed at either MYC (Pearson's Correlation = 0.102, $P = 0.114$) or the drone congregation area DCA (Pearson's Correlation 0.068, $P = 0.458$, N = 119).

Confirmed mating flights (N = 23) had a mean duration of 22.11 ± 5.48 min and were of significantly longer duration (Z= -3.41, $P = 0.001$, Wilcoxon signed rank test) than same day non-mating fights. All flights above 18 minutes were therefore assumed to be putative mating flights. Twenty three of the thirty putative mating flights could be confirmed by the presence of a mating sign. There was a positive correlation between the number of putative mating flights and mating success ($P = 0.012$, Spearman's rho = 0.51). Ten queens started laying after only one mating flight, eight after two flights, and two after three flights. Confirmed mating flights occurred at temperatures ranging from 17.1 to 21.2 ºC.

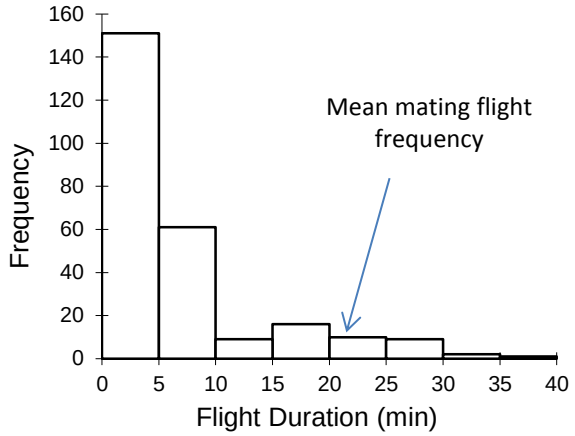

Figure 2. There was a skewed distribution in flight duration. A total of 251 flights were observed of which around 150 were of short duration (<5 minutes). Mating was confirmed by the presence of a mating sign in the returning queen on 23 occasions. The mean duration of mating flights was 22.11± 5.48 minutes

Twenty of the thirty (66%) experimental queens mated and developed self-sufficient colonies (A= 8/15; B = 12/15). One colony failed in each batch (1 and 2) as the virgin did not emerge successfully. Twenty eight virgins were therefore observed to undertake orientation flights. Three queens from batch (1) were lost, and an additional three failed to mate (thus becoming drone layers). Two queens from the second batch (2) failed to mate.

Of the queens that mated successfully, there was minimal difference in the observed (N_o) and estimated (N_e) mating frequencies (means of 7.35 ± 2.06 and 7.34 ±2.22 respectively; Table 1) indicating that the sample size was adequate to capture paternal contributions. The observed mating frequencies fell within the 95% confidence internal of the effective mating frequencies (mean m_e = 6.50 ± 1.91; Table 1) in most cases, indicating that drone contibutions were of relatively equal proportions. Colonies 3, 21, 29 and 30 were exceptions due to skewed drone contributions.

The summer of 2010, was exceptionally cold and wet in Wales. It rained on 28 out of the 46 days of the experimental period at MYC and the total rainfall recorded was 229 mm. It was wetter and slightly warmer during the first half of the experiment (1st to 22nd of July). Mean afternoon temperature was 18.9 ±1.78 ºC and a total rainfall was 153mm was recorded during this period. The mean afternoon temperature and total recorded rainfall between July 23rd and August 15th was 17.30 ±1.44 degrees ºC and 67mm respectively (Fig. 1). Our data revealed no correlation between mating success and temperature (*P* = 0.127, Spearman's rho = 0.35, N = 20)

Table 1. Mating Success of Experimental queens

Colony ID	Sample size	Observed mating frequency (N_0)	Estimated mating frequency (N_e)	Effective mating frequency (m_e)	95% CI of m_e
2	36	7	7.03	6.78	0.89
3	32	5	5.00	3.36	0.58
4	35	5	5.00	4.55	0.53
7	40	10	10.16	9.30	1.42
10	40	8	8.04	6.68	0.99
11	37	9	9.12	8.39	1.30
14	38	9	9.11	6.34	1.26
15	37	10	10.23	9.04	1.55
16	27	9	9.47	8.50	1.84
17	37	9	9.12	8.62	1.30
18	24	4	5.00	4.26	0.56
21	36	5	5.00	3.69	0.51
22	38	5	5.00	4.63	0.48
23	36	5	5.00	5.57	0.51
24	38	9	9.11	8.38	1.26
26	31	6	6.02	5.97	0.81
27	40	7	7.01	6.78	0.81
28	38	9	9.11	8.20	1.23
29	36	10	10.26	7.01	1.60
30	38	6	6.01	4.86	0.65
Mean	35.7 ± 4.2	7.35 ± 2.06	7.34 ± 2.22	6.50 ± 1.91	1.00 ± 0.4

The mating apiary (MYC) was located in a sheltered narrow valley. The mean wind speed recorded at 15 m above the ground between the 24th of July and the 12th of August was 0.31 ± 0.39 ms^{-1}. The mean wind speed recorded at the DCA (approx. 7 m above ground) during this same period was 1.85 ± 1.39 ms^{-1}. The DCA was in an open field and exposed to the prevailing south easterly winds. Wind speeds were also generally higher during periods of dry sunny weather. Queens mated when constant winds of 3.13 ms^{-1} were being recorded at the DCA. Indeed, queens returned from mating flights when the DCA station was consistently recording winds of between 3.39 and 3.80 ms^{-1}, and with gusts up to 5.28 ms^{-1}. No correlation was detected between mating success and wind-speed at either the mating apiary (MYC) or the DCA (P = 0.369, and 0.366; Spearman's rho = 0.22 and 0.31; N = 19 and 11 respectively). The mean afternoon (1pm to 6pm) temperature at the mating yard was 18.09 ± 1.84 ºC.

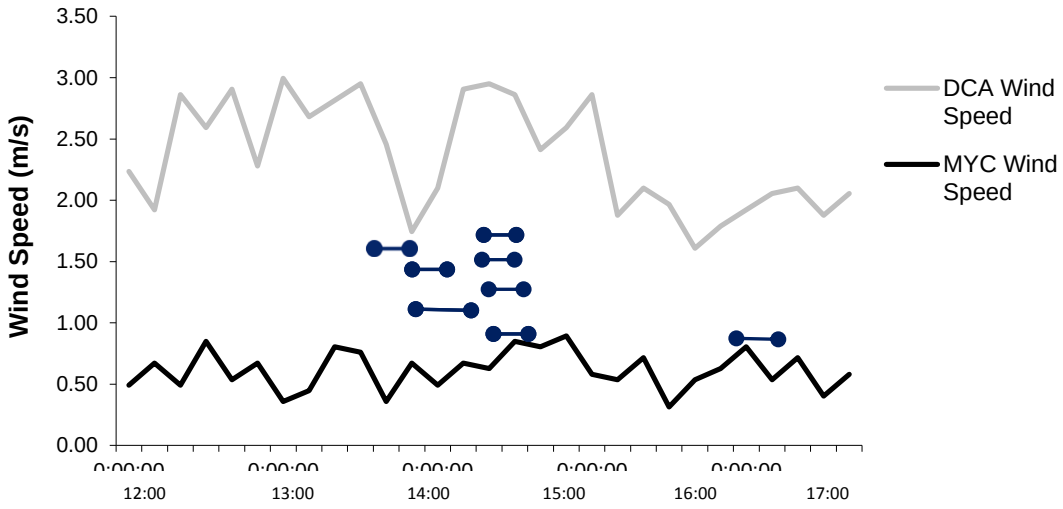

Figure 3. A figurative representation of queen mating flight times and duration in relation to wind speeds (logged at 5 minutes intervals) recorded at the mating apiary (MYC) and at local drone congregation area (DCA) between 12:00 and 17:00 hrs. on August 8th, 2010. Queen flight events are represented by the horizontal lines. No correlation was detected between mating success and wind speed at either the DCA or mating apiary.

Discussion

Our data suggest that both mating behaviour and success were affected by the weather. Queens undertook many flights of short duration. Confirmed mating flights were far less frequent and of significantly longer duration. Favourable mating opportunities were brief and compressed by extended periods of rain and low temperatures. Of the twenty-eight

queens that were observed flying, three did not return, and five failed to successfully mate. Our data detected no correlation between observed number of pairings (mating success) and the average temperature and wind speeds recorded during the mating flight period. Of the queens that successfully mated, 90% initiated oviposition after only one or two flights, suggesting that they might have modulated their nuptial flight activity in response to climatic conditions. A significant correlation was observed between mating success and number of confirmed mating flights undertaken. Mating was also delayed in all cases, with queens mating on average with seven drones (range 4-10). Similar mating frequencies were obtained by Krause et al. [36] who suggested that environmental conditions had limited the mating success (range 3-13) of *A. m. carnica* experimental queens. Similarly, it was demonstrated that queens mated under island conditions have lower mating frequencies than those mated on the European mainland [35, 37]. Bees considered well adapted to northern climates (*A.m. mellifera* and *A.m. carnica*) will commonly mate with 15 or more drones when conditions allow [22].

Queens that undertook multiple mating flights were likely to have greater mating success. It is not clear if queens 'decide' to undertake additional flights based on the copulation success of previous flights. Tarpy and Page [32] observed no difference in the mating success of queens that naturally started oviposition after one nuptial flight, and those that attempted but were prevented from taking a second flight, and in which oviposition was stimulated by CO_2 anaesthesia. They concluded that queens have little behavioural control over nuptial flight frequency. In contrast, Schlüns et al. [38] found a significant difference in the mating success of similarly categorized experimental queens. They concluded that queens can adjust their flight frequency based on the mating success of the previous flight, and that number of copulations stimulates oviposition. They note that queens might have a variable 'threshold' that is responsive to environmental queues. A lower threshold might be expected during periods of poor weather due to the additional risk involved. Franck et al. [39] also suggest that queens might adjust their mating flight frequency in accordance to prevailing climatic conditions. Our observations support this postulate as queens took numerous flights of short duration that were correlated with high humidity and low temperature. Similar behaviour was described by Lensky and Demter [17] who noted that queens took more flights of short duration during colder temperatures (15-20 ºC) and wind speeds between 2.6 and 2.88 m/sec. We found no correlation between flight duration and wind-speed, but queens mated successfully in constant winds up to 3.8 ms^{-1}. These

observations are also consistent with earlier research that suggests mating occurs only when wind speeds are less than 4-5 m/s [17].

The mechanisms driving extreme polyandry in honeybees are not fully understood [36]. At the colony level, genetic variance hypotheses are favoured , although little increase in intra-colony diversity occurs after six matings [22] suggesting minimal adaptive advantage at higher mating numbers. Alternatively, the sperm limitation hypothesis proposes that queens must mate multiply to offset premature sperm deficiency [14]. Woyke demonstrated that the number of sperm stored in the spermatheca reaches capacity after 8µl of semen is inseminated. Work by Schlüns et al. [38] on sperm number and mating frequency in naturally mated queens corroborated Woyke's earlier instrumental insemination work. It appears that naturally mated queens need only mate with 10 or so drones to acquire a lifetime volume of sperm.

Based on research by Woyke [14], and Zmarlicki and Morse [15], delayed mating is considered detrimental to queen vitality by beekeeping experts [9,10]**.** None of the experimental queens in this study managed to mate within this optimal 14 day window. The experimental period was characterized by long periods of cool overcast conditions, including periods of extended daily rainfall (hence high humidity) (Fig. 1). The weather clearly delayed mating, with queens not mating until they were 17 days old on average (min 14 - max 23; note that this is an approximate age since actual date of emergence in the mating nuclei was not known). Assuming time to oviposition was not delayed, our queens would have started laying approximately six days later [10] at an average age of 23 days (range 17 -26 days), one week later than a recently reported species mean [10]. Previous research by Szabo et al. [40] and Skowronek et al. [41] had reported mean age of oviposition as 10.6 and 10 days respectively, and the former suggest a relationship between max daily temperature and time of oviposition. Guler and Alpay [12] reported a significantly longer pre-ovipositon period for *A. m. carnica* (15.04 ± 0.23 days) compared to five genotypes of *A. m. liguistica* and four regionally distinct groups of *A. m. caucasica*. They found no significant loss in production due to delayed mating. Our data revealed no correlation between approximate age of first mating, and ultimate mating success and we cannot say whether delay in mating affected the ultimate mating success of our queens. It is well known that instrumentally inseminated queens that are anesthetized by carbon

dioxide to stimulated egg-laying, take longer to start laying when compared to naturally mated queens. No real difference in performance between these two groups is claimed [9].

Assessing the mated health of MYC queens

As previously noted, colony relatedness decays only minimally above a queen mating frequency of six. Accordingly, fourteen of our twenty experimental colonies should benefit from 'adequate' levels of intra-colony genetic variance. However, since the semen from ten or so drones is required to fill a queen's spermatheca to capacity with sperm, it appears that approximately half of our mated queens may be inadequately mated and have a shorter effective laying lifetime as a consequence. No diploid drone production by experimental queens was noted. We observed solid brood patterns which suggested that sufficient numbers of sex alleles were available in the population.

Summary

Our data suggest that queen flight behavior was influenced by environmental conditions and that queens might modulate mating flights according to environmental cues. Approximately twenty percent of our flying queens failed to mate, and most 'successful' queens undertook only one or two mating flights. Mean mating success was accordingly low. It is possible that these results are normal for the area and reflect the fact that Wales is on the fringe of the natural range of the western honeybee, and commonly experiences high rainfall and cool temperatures due to its geographic location and elevated topography. However, conditions during recent breeding seasons have been especially poor and the observed results might be due to combined effects of location and medium to long-term shifts in climate trends. We cannot rule out the possibility that one of the main causes of premature failure of Welsh honeybee queens is suboptimal environmental conditions during the breeding period and consequently inadequate mating.

Materials and Methods

Queen rearing and experimental set-up

The Cloake board queen rearing method was used during July and August 2010. Briefly, this involved grafting one day old larvae into artificial queen cells and introducing them to the top box of a two story colony. The queen and unsealed brood are isolated in the bottom box and separated from the rest of the colony by a solid board (Cloake board). The top box is supplied with pollen, nectar, and emerging brood and crowded with young nurse

bees. These manipulations are designed to mimic natural queen rearing conditions. Bees in the top cell raising box experience overcrowding (which induces swarming tendencies) and are missing a queen (which induces the colony to build emergency queen cells). Two batches were raised in an effort to broaden the environmental variance, and to distribute the observation/recording load. All experimental queens were grafted from the same mother hive to limit genetic variance influencing mating success.

Each queen was allowed to emerge into a modified six-frame polystyrene nucleus hive located at the experiment apiary in Maes-y-Coed (MYC), Ceredigion, Wales. These nuclei incorporated a plexi-glass entrance tunnel gated at front and rear with a removable section of queen excluder. The tunnels were designed to cover the hive entrances (approximate dimension 5 cm x 3 cm) and were approximately 15 cm long, 12 cm wide, and 10 cm deep. A queen exiting a hive was allowed access to the observation tunnel, but was prevented from undergoing flight by a gate at the front end of the tunnel. When a queen was observed in the tunnel, a rear gate was inserted to essentially cage the her. The front gate was then removed to allow access to the open environment and possible flight. Departure and return times were recorded. Returning queens would enter the entrance tunnel, and the front gate would be closed. Access into the main hive body would still be restricted at this point by the in-place rear gate. Return flights could then be confirmed, and queens could be visually checked for signs of mating before being allowed access into the hive. The first batch of queens were monitored between the 12th and 26th of July, and the second batch between the 30th of July and the 16th of August. Daily monitoring occurred between 10:00 am 6:00 pm.

Queens utilize visual cues to orient onto their home nest entrance. To aid queen orientation and to reduce the likelihood of drifting, colonies of differing colours were paired and set side-by-side with entrances facing in opposite directions. Colony pairs (N = 7 per batch) were also spaced four to five feet apart and arranged into a square formation. Queens were introduced as ripe (10 day old) cells, and emerged within a day or two into the colony environment. The post-emergence period is the most critical time affecting ultimate queen (and hence colony) success, and colonies are particularly sensitive to disturbance during this time. Consequently, the experimental colonies were not disturbed during this time, and queen were not individually marked.

Queens were allowed to take multiple flights. Flight time and duration were noted and climatic conditions recorded using iROX PRO-X 2 weather stations. Weather data, including temperature, rainfall, wind-speed and direction, were recorded every five minutes during the experimental period.

A drone congregation area (DCA) just under 1km away from the mating yard was located during this period. In contrast to the mating apiary, which was situated in a sheltered, shaded valley, the DCA was located in a field up off the valley floor and was exposed to all aspects of the prevailing weather. A second similar recording unit was therefore installed at the local drone congregation area (DCA) before the August batch of queens emerged.

Statistical Analyses of climate and mating success data

We investigated the influence of apiary temperature and wind speed on flight duration, and tested the hypothesis that mating flights are significantly longer in duration than non-mating localized flights. The temperature data were normally distributed but the distribution of the flight duration data were visually skewed. They were therefore log transformed and tested for normality using the Kolmogorov-Smirnov test.

We also tested for a correlation between mating success and number of putative mating flights, apiary temperature, and wind speeds at both the mating apiary and DCA. The mean temperatures and wind-speeds recorded during the flight periods (data logged every 5 mins.) were used in these instances. In addition, averages across number of flight were used when queens had undertaken multiple mating flights. Non-parametric methods (Spearman's) was preferentially used to investigate possible effects on mating success due to relatively low sample sizes. *Sampling and genotyping experimental brood*
Forty pupae per colony were sampled approximately six weeks after the queen cells were introduced to eliminate the possibility of "phantom" genotypes drifting in with worker bees from other colonies. Sealed brood was sampled at the purple- or post purple-eye stage and individually stored in 100% ethanol. DNA was extracted from equal volumes of pupal leg and thorax tissue using a modified version of the 96 well plate protocol described by Lagisz et al. [42]. The extraction protocol was performed in 1.5ml tubes and the reagent volumes adjusted accordingly. The extracted DNA was quantified using a NanoDrop nd100 spectrophotometer and each sample was diluted to 50ng/µl for genotyping.

Genotyping

Seven microsatellite markers (Table 2) were amplified in a single multiplexed reaction and genotyped on an ABI 3130xl Genetic Analyzer. Markers were amplified in a single 10ul multiplexed reaction consisting of 50 ng of DNA, 0.75 X Qiagen multiplex PCR solution, 2.5 and 0.25 pM of reverse and forward primer mixes respectively, and 2.5pM of ABI Hex-, Pet- and Ned- and 5.0pM of Vic-labeled primer (Table 2).

Table 2. Microsatellite loci used in this study

Locus	Unified locus ID	Authors	Number of alleles	Heterozygosity	Label	Accession number
A7	Am005	Estoup et al. 1994	11	0.807	PET	AJ509236
A14	Am406	Solignac et al. 2003	14	0.825	VIC	AJ509239
A29	Am014	Solignac et al. 2003	22	0.917	VIC	AJ509245
A79	Am046	Baudry et al. 1998	12	0.801	FAM	AJ509277
A107	Am056	Solignac et al. 2003	24	0.922	FAM	AJ509287
A113	Am059	Estoup et al. 1995	11	0.800	NED	AJ509290
Ap14	Am068	Solignac et al 2003	11	0.8125	NED	AJ509305
		Mean	**15±5.60**	**0.841±0.05**		

Polymerase chain reactions were performed on a DNA engine Tetrad 2 thermocycler (BIO RAD) using the following cycling parameters: 95°C for 15 min, followed by 13 cycles of 94°C for 45 sec, 55°C for 45 sec and 72°C for 45 sec, and then 25 cycles of 94°C for 45 sec, 52°C for 45 sec and 72°C for 45 sec. The profile was terminated with a 30 min extension at 60°C. Reaction products were visualized on an ABI 3130xl Genetic Analyzer and the data were analyzed using Genemapper (ABI).

Parentage analysis

We used the program Colony 2.0 [43] to determine the number of full-sibships (equal to the number of contributing drones) in each colony. We ran each data set up to five times with different seed numbers to ensure consistency of results. The effectiveness of Colony can be limited by the availability of sufficiently informative unlinked loci since the probability of not detecting a unique paternal genotype decreases with increasing number and variability of markers (non-detection error). Based on available population level allele frequency data derived from a broad sample (Table 1) we limited the non-detection error (d_p) to 2.95 x 10^{-6} [44]. We made the assumption of equal sex-specific allele frequencies so that:

$$d_p = \prod\left(\sum {q_{ij}}^2\right) \qquad (1)$$

where q_{ij} is the frequency of allele i at locus j. Our markers provided sufficient power to detect patrilines hence it is unlikely that discrepancies due to sex-specific allele frequency differences would affect our results.

The observed number of patrilines (N_o) in a finite sample can underestimate the actual number (N_e) due to non-sampling error. We calculated the estimated number of patrilines (N_e) following procedure outlined by Schlüns et al. [38]:

$$N_o = N_e - [N_e(1 - 1/N_e)^n] \qquad (2)$$

where N_o = number of observed matings as determined by Colony, and n = the number of colony progeny sampled. N_e = estimated number of matings and was determined by iterating N_e for (N_o). The degree of discrepancy between the observed and estimated values provides an indication as to the adequacy of sampling. This calculation assumes equal number of individuals per sub-family; an assumption that is unlikely to be true. We therefore also present the 95% confidence interval around the effective paternity (m_e) as a method to account for sampling error [25]. Effective paternity was calculated using an unbiased estimator from Nielsen et al. [45] and provides a weighted value accounting for disproportional paternal contributions

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Author Contributions

Conceived the experiment and wrote the paper: IW and AM. Performed the experiment, collected the data and designed the microsatellite multiplex system IW. Analyzed the data: IW

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Chapter 3

Selection on Phenotype

3.1 Introduction

An individual's phenotype (its observable characteristics) depends upon both its genetic makeup and on the environment in which it is found (Futuyama, 1998). Likewise, a population's variance in phenotype depends upon the genetic makeup of the individuals within it and on the influence of its environment. This relationship, for individuals and populations alike, can be expressed as;

$$V_P = V_G + V_E$$

Literally we can say that phenotypic variation (V_P) is equal to genetic variation (V_G) plus environmental variation (V_E). Genetic effects (V_G) can be separated into three parts of which additive effects are the most important from a breeding perspective. Additive genetic effects equal the sum of average effects of all the genes an individual carries (Rinderer, 1986). The other two genetic influences are the non-additive interactions between different alleles at the same locus (dominance effects) and the effects on phenotype of interactions between different loci (epistasis). Additive genetic influences are most important from a breeding perspective since they affect resemblance between relatives.

Heritability (h^2) is another important and related quantitative genetic property. It is defined as the ratio of additive genetic variance to the total phenotypic variance, expressed mathematically as;

$$h^2 = V_A/V_P$$

Heritability is important as it gives an indication of how responsive different characters are to selection, and is as such, the proportion of total phenotypic variance that is attributable to additive effects. It not only depends upon the property of a specific character, but is also sensitive to the influence of specific population and environment characters (has V_P in the denominator). Heritability depends therefore on specific population parameters (e.g. size). This is relevant to breeding since small closed populations under selection will have lower heritability's that larger randomly mating ones. Also, populations with differing allele frequencies will respond differently to selection.

Fecundity, industry, resistance to disease and temperament are commonly cited as primary performance qualities for managed populations of honeybees (Adam, 1987), and in general, these were the qualities targeted for improvement by the WWBBP. Related character traits have all been shown to be heritable (Table 3.1). The goal was to improve

the mean population phenotype by altering, through selection, the underlying related genetic makeup of the population.

Table 3.1. Published heritability's (h^2) for character traits relating to a) brood production b), honey foraging c), defensiveness d), and *varroa* resistance.

	Character Trait	h^2	
a)	Brood (yearly average)	0.90	Banby, M. A. el, 1967
	Brood (6 weeks before nectar flow)	0.30-0.41	Vesely and Siler, 1963
	Brood (winter)	0.76	Soller and Bar-Cohen,1967
	Brood (spring)	0.33	Soller and Bar-Cohen, 1967
b)	Total honey	0.57	Soller and Bar-Cohen, 1967
	Honey yield	0.16-0.19	Vesely and Siler, 1963
c)	Time to react to Isopentyl Acetate	0.68	Collins, 1979
	Time to react to Isopentyl Acetate	1.28	Collins et al., 1984
	Time to react to moving target	0.69	Collins et al., 1984
d)	Grooming behaviour (African Honey Be	0.71	Moretto et al., 1993
	Hygienic behaviour	0.65	Spivak. 1996
	Mites per 100 bees	0.28 ± 0.56	Harbo and Harris 1999
	Mites per 1000 bees	0.01 ± 0.46	Harbo and Harris 1999

3.1.1 Breeding for Productivity

As improving production potential (a heritable trait) was a key project objective, I tried to develop an approach that could be used to compare the foraging efficiency of colonies. This was not a straightforward endeavor since various complicating factors must be considered when comparing young (1st season) colonies. These nucleus colonies were constructed under field conditions; hence they were not strictly standardized during construction. There was likely variation in the amount and age of brood and bees used for example, or frames transferred during construction may have bees in some way diseased (e.g. carrying chalkbrood spores-glossary). Such factors can influence the developmental rate of the colony. Nevertheless, first season assessments were conducted on the heather and were designed to highlight colonies with potentially superior productive traits.

I considered comparing colonies for production potential as follows. It seems hypothetically possible (assuming all other contributing factors to be equal) that a colony with fewer but more efficient foraging bees might weigh more than a colony with greater numbers of less industrious bees (i.e., less efficient at gathering nectar and pollen). One might therefore observe a lack of correlation between colony strength and weight change during a nectar flow if a population has high variance in foraging efficiency. By recording weight change, one can eliminate confounding factors such as unequal weight of colony woodenware. Another possible way to identify better producing colonies might be to regress the end of season colony weight on frames of bees. If a correlation is observed (as might be expected) between these two variables, an expected curve can be generated. Colonies with above average weight, in relation to number of bees (i.e. above the curve) would be highlighted as better performing.

3.1.2 Selecting for varroa *mite resistance*

A conscious decision was made not to select for specific heritable mite resistant/tolerant behaviors (e.g. hygienic behaviour, *varroa* sensitive hygiene VSH, grooming). Bee strains with enhanced VSH qualities have been developed by federally funded establishments in the USA, but such work is too demanding of time and effort (selecting for VSH particularly so) for small scale operations. There is also a cost incurred by focusing only on a single resistance specific trait. Overly vigorous VSH colonies can retard brood development and have reduced production potential, and may make them more susceptible to cold weather, for example. Since so much goes unnoticed in the honeybee colony, it is likely that many subtle and currently unrecognized mechanisms confer varying degrees of colony level tolerance. Independent bee operation can enhance these population traits by each year breeding only with the strongest treatment free survivors.

3.1.3. Other considerations relevant to honeybees

The eusocial structure of the honeybee colony complicates the process of selection and breeding. For example, for certain traits (e.g. honey production) we are assessing the performance of an individual (queen) for breeding based on the performance and behavior of a collective group from a different caste (the workers). Although queens and workers that are raised in the same colony are genetically similar, they develop in different environments. Similarly, we may select drone- producing colonies based on the hygienic

performance of the worker population. In addition, the relatedness of individuals within a colony varies to a greater or lesser extend depending on the number of contributing patrilines. Without artificially inseminating queens (Instrumental Insemination) one can never therefore be certain of the paternal source when selecting queens.

Wales is on the north-western margin of the native distribution of the honeybee; hence consideration was given during breeder selection to regionally-appropriate characteristics. For example, the queen must be sensitive to environmental cues to control brood rearing since the region can experience prolonged periods of inclement weather at any-time of the year. Colonies must be able to rapidly expand and forage efficiently during periods of good weather, and display frugality during periods of dearth. The strongest of surviving overwintered colonies were compared in the spring 2011. Potential breeders were assessed by comparing colony condition (strength and general health) and general organization of the brood nest. Choice colonies had nests comprising of ample sealed and unsealed brood surrounded by consecutive arches of pollen and nectar/honey respectively.

Apiary location can also greatly influence colony performance. Factors such as elevation, aspect, shelter and availability of forage will all affect colony performance. Breeders were therefore selected from different overwintering locations in an effort to accommodate for differences due to environmental influences. The breeder daughter (test) colonies were similarly distributed to compare the performance of sister queens in different environments. Wales has recently experienced a series of poor summers, and accounting for confounding environmental effects on monitored traits was expected to be challenging in an applied setting. I nevertheless expected some observable change due to selection in the population during the experimental period. In particular, I expected greater uniformity as the number of breeders was effectively dropped from 8 to 4 between years. That is, as genetic input was constrained across generations, I hypothesized a detectable and concurrent reduction in colony level phenotypic variance. Of the characteristics I monitored, colony strength and temperament were considered to be more likely to adaptively respond to selection. Mite counts per colony is not a strongly heritable trait, and detectable reductions in population level variance was considered less likely.

3.2 Methods

3.2.1 Grafting and raising queen cells

Frames with brood of appropriate age were removed from selected breeders, and marked with coloured press-pins in the field. These were placed in nuc-boxes (with bees to help maintain brooding environment) for transportation back to the grafting shed.

A variation on the Cloake board method was used for queen rearing. A single cell raising unit consisted of two stacked Modified Dadant (MD) brood boxes. Strong colonies were used as raisers since high bee density (particularly young nurse bees) is needed. The colony queen was isolated in the bottom box along with unsealed brood, stores and empty frames. The remaining brood, stores and empty drawn comb were placed in the top box which was separated from the one below by a queen excluder (glossary). A single entrance located at the front of the bottom box is provided at this stage. Each cell raiser was copiously fed with syrup and pollen, particularly in the absence of strong natural nectar flows, and left for up to eight days in preparation for receiving grafts. By then, many young nurse bees would have emerged in the top box and any remaining brood in this part of the hive would be sealed.

In preparing the colony to accept grafted larvae, all the top box frames were shaken of bees and checked for natural queen cells. It is vital that no queens (virgin or mature) or queen cells be present in the cell raising box (top box in our method). Any suspicious queen cell structures were removed. I also ensured that the colony queen was still in the bottom box and had not accidently passed into the top. The bottom box and entrance was then turned through 180°, and a solid board (the Cloake board) was placed on top before the top box was replaced. A solid board now separated the two colony halves, isolating the bees above from the effects of the queen down below. The board also provides a new front entrance into the top portion of the hive only. Conditioned front oriented bees leaving the hive via the rear bottom box entrance would then return into the top. These manipulations increased bee density in the top box in preparation for cell building.

Theoretical modelling by Moritz (1984) suggested that inbreeding could be limited to acceptable levels for 10 generations if a minimum of 8 breeder queens per year were used. Eleven breeders were therefore selected in the spring of 2011 from a source population consisting of 2010 and older colonies (Table 3.1a). The breeding program logistics proved challenging with this number of breeders. In addition, some breeders were not well

represented in the final daughter cohort since the program could only establish a certain number of new colonies. Consequently, the full genetic potential of some breeders was not well represented (exposed) in the next generation. An executive decision was made by the company director to use only four breeders in 2012 (Table 3.1b). Again each breeder from each year did not contribute equally to the following generation due to unequal survivorship of daughter queens. Consequently, the 'effective' number of breeders used in 2011 and 2012 was 8.3 and 3.5 respectively.

Table 3.1a Selected breeders 2011

Breeder ID	Y.O.B	Location	Daughters	Heather
Anwen 1	2010	Galspwll	6	6
Catrin 1	2010	Glaspwll	16	11
Branwen 1	2009	Mathafarn	5	4
Carys 1	2010	Abercegir	13	8
Llinos 1	2010	Morben	13	9
Marged 1	2010	Morben	6	1
Nia 1	2010	Abercegir	20	10
Gwenllian 1	2009	Pennal	12	2
Dwynwen 1	2010	Abercegir	11	2
Lucy29 1	2009	MYC	15	7
Sioned 1	2009	Hendres.	1	1
		Sum	**118**	**61**

3.2.2 Making nuclei colonies

Nucleus colonies were made by taking 'splits' (see glossary) off strong survivor colonies. Each new split was provided with two frames of sealed brood placed in the middle of a six frame box. A frame of (honey/pollen) and drawn or undrawn foundation was added to each side of the brood. Additional bees were shaken into the box to ensure that developing brood, and the soon to be added queen cell, would be maintained at an adequate rearing temperature. Entrances were closed during construction so that the colonies could be moved into one of two established mating apiaries in the isolated Glaspwll valley (an additional location was used in 2012). The colonies were arranged in pairs on hive stands with their entrances oriented in different direction. This arrangement helped the relocated bees orient onto their home colony after release. The bees were released in the evening (post flying hours) after a single ten day old queen cell (day or so from emergence) had

been placed between the two brood containing frames. Procedure time frame was determined by the developmental rate of the queen bee (Fig. 3.1).

CALANDER		DAYS AFTER EGGS ARE LAID	DAYS AFTER GRAFTING
June 1			
June 2			
June 3	-	1-	
June 4	-	2-	
June 5	-	3- Eggs Hatch	
June 6	-	4- Grafting (24-36hr old larva)	
June 7	-	5- Check if bees have accepted and started building cells	1
June 8	-	6-	2
June 9	-	7-	3
June 10	-	8-	4
June 12	-	9- Cells capped	5
June 13	-	10-	6
June 14	-	11-	7
June 15	-	12-	8
June 16	-	13- Remove queen cells from starter/finisher colony	9
June 17	-	14- Add queen cells to pre-prepared nucleus colonies	10
June 18	-	15-	11
June 19	-	16- Queen emerges	12
June 20			
June 21			
June 22			

Figure 3.1. The rearing timeline is determined by the development biology of queens. Developing cells were normally removed from the building colonies nine days after grafting, and introduced to queenless nuclei within 24hrs. They were incubated at 37˚C in the interim.

Table 3.1b Selected breeders 2012

Breeder	Y.O.B	Daughters
Anwen 2	2011	10
Carys 2	2011	21
Catrin 2	2011	9
Llinos 2	2011	18
	Sum	**58**

The nucleus colonies were left undisturbed for a month to give successfully mated queens time to mature and start egg-laying. Colonies are sensitive during this period since the post-emergent queens have entered the most precarious developmental phase. They have only a brief window to successfully mate, and they must leave the safety of the hive to do so. Mating on the wing they must navigate through unfamiliar territory and possibly brave poor weather and predation to do so.

3.2.3 Measuring colony strength and foraging efficiency

Various measurements gave an indication of colony strength. Individual frames were visually inspected during colony development and scored for brood strength (Fig 3.2). Each side of a brood bearing frame was scored for brood density (range 1-4) so that a whole frame could receive a maximum score of 8. Individual frame scores were tallied for an overall colony score. The 'frames with bees' index was a simple description of how many frames the bees were actively occupying and utilizing. A homemade portable scale was used to weigh single box hives in the field (Fig. 3.3).

Comparative weight gains during periods of nectar flow were used in an effort to identify colonies with foraging with above average efficiency. Colonies were weighed before and after periods of good weather while on the heather, and measurements were taken in the evening when most of the flying bees were back in the hive. The goal was to assess foraging efficiency by comparing colony strength to rate of weight gain during nectar flows. Weather conditions on the heather moors were uncharacteristically poor during the 2012 season. Weight gain comparisons were not possible this season since the bees were not able to forage for suitably prolonged periods. Heather quality was low and colonies weight began dropping towards the end of the season.

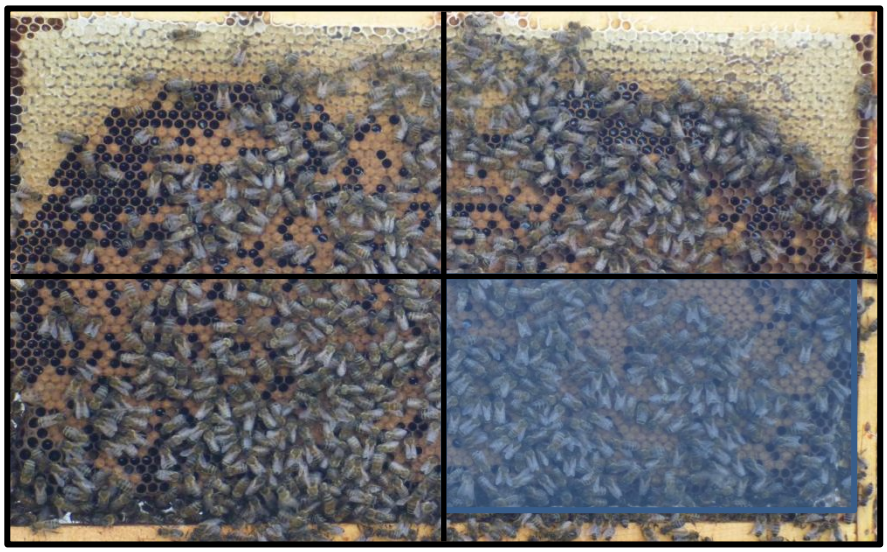


Figure 3.2 Brood (sealed and unsealed) was assessed by visual inspection. Each side of a brood-frame was divided into four separate sections. The half frame above was scored 2.5. A maximum frame score of 8 is theoretically possible

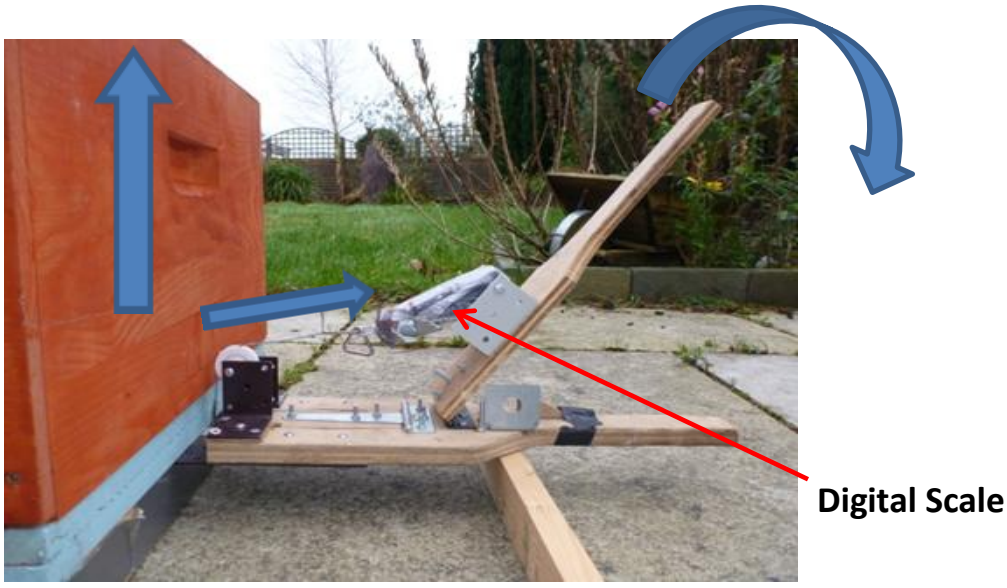


Figure 3.3 An image of the portable scale designed to weigh single box hives. Force is applied to the diagonal bar (curved arrow) and the resistance required to raise the box is recorded on a digital scale. This value is doubled to give an approximate colony weight.

3.2.4 Varroa mite counts

The sugar shake method (Macedo and Ellis 2002) was used to monitor for *varroa*. Approximately 300 bees (approx. 150ml marked on clear jar) were shaken off a frame of brood (after ensuring that the colony queen was elsewhere) and placed into a jar covered with a woven wire 8 mesh cover lid. Using a hive tool tip, approximately one table-spoon of dry powdered sugar was added, and the jar left for one minute. The jar was then shaken vigorously over a white enamel bowl containing water. The dark dislodged *varroa* would be visible floating on the water against the light background.

3.2.5 Measuring colony temperament

Temperament was assessed subjectively in 2011, and quantitatively in 2012. Colonies were subjectively given a score (range 1—5) based on behavior. Particularly defensive (i.e., quick to begin stinging) and 'runny' colonies were scored the lowest, and docile calm colonies the highest. A more quantitative method was adopted in 2012 because the bees were defensive from the beginning of the season. Although environmental conditions were particularly bad this season (possibly a significant contributing factor) there was concern that genetic influences due to breeding were affecting population temperament. Colony temperament was investigated using similar methods to those described by Guzman-Novoa et al. (2004). Immediately after opening the hive, a piece of black leather (5cm square) that was stapled to the end of a wooden wand was lowered to within approximately 10cm of the frames. The leather antagonist was rhythmically lowered and raised once a second for 45 seconds. The number of stingers in the leather was used as a quantitative assessment of colony defensiveness. The temperament of all the experimental daughters were qualitatively assessed as described above after the colonies had matured and migrated to the heather. All colonies were tested on the same day and under similar weather conditions. A control apiary comprising of 2011 and older colonies from an unknown mix of breeders (N=26), representing the unselected background phenotype was also tested a few days later under similar weather conditions.

3.2.6 Data analysis and colony comparisons

A z-score index was used to compare colonies of different age (n = 3) classes in 2011, and a similar approach was used to compare the daughter colonies of different breeders (n = 2) in 2012. Z-scores allow comparison among cohorts in units of standard deviations where:

$$colony\ z\ \ score\ per\ trait = \frac{colony\ score - mean\ cohort\ score}{cohort\ standard\ deviation}$$

Three traits were used in our initial colony comparisons, and separate trait scores were summed for an overall colony score. The three factors were: *varroa* count, weight change of colony during a period of nectar flow (2011) or during the whole period on the heather (2012), and temperament (Table 3.2). Each factor was prioritized and its influence on the final colony score accordingly weighted. Production and temperament were given greater weight in this model. Although *varroa* counts provide good indication of infestation rates, they provide no information about a colony's behavior and expression of actual mite resistant traits. Mite count is therefore only slightly heritable (Harbo and Harris, 1999) it was given reduced weight in my model (Table 3.2).

Table 3.2. This table presents an example of a final z-score calculation (2011). A z-score was calculated for character trait for each individual.

Colony ID		*Varroa* Count	Weight Change	Temperament	Final Score
Anwen 1.1	Z-score*	-1.02	-1.09	0.18	
	Weight[$]	X -10	X +30	X +20	
	Score	10.20	-32.79	3.64	**-18.96**[#]

*This score allows one to compare traits among cohorts in units of standard deviations where z = (colony score-mean cohort score/cohort SD).
[$]Each trait can then be weighted (negatively or positively) according to economic importance for example.
[*]Individual trait score are tallied for a final sum score

I used qualitative data to investigate potential breeder (n=4) and grafting cohort (n=2) effects on the temperament and defensiveness of colonies in 2012. These daughter colony queens were grafted from the same four breeders during two separate rounds of grafting. A Kruskal-Wallis test was used to test for difference in temperament between the two grafting cohorts; i.e., was there a difference in the defensive temperament of colonies headed by queens raised from the same four breeders, but mated at a different time, and more importantly, location. All colonies were tested under similar weather conditions on the heather.

Preliminary assessments, standardizing for colony age (2011) and breeder (2012) using z-scores, identified the thirty top performing hives each year. These were moved into two overwintering apiaries in mid-Wales for possible selection as breeders the following year. Accumulated colony specific data were forwarded to the commercial partner.

3.2.7 A comment on monitoring adaptive change

The WWBBP is a long term project aiming to improve the resilience and production potential of its bee stock. The program is in the early stages, having currently gone through two rounds of breeding. It is a field operation exposed to the vagaries of the weather. Environmental conditions have been uncharacteristically demanding during recent years, and demonstrable evidence that selection is having a positive effect on desired character traits is lacking. A limitation of this field approach is the lack of suitable control populations, to which adaptive shifts in the test group could be compared. In order to demonstrate an effect due to selection, I compare trait variances across generations, under the assumption that it should shrink due to greater genetic uniformity resulting from selection.

A total of 118 new colonies were established in the spring of 2011 (Table 3.1a) and approximately seventy-five percent mating success was achieved. The most promising looking colonies (N = 61; based on colony strength and condition at time of transfer from nucleus boxes into full bodied hives) were selected for a 60 mile migration to the heather moors during the first 2 weeks of August. Each of the three age classes was represented by 21, 28 and 12 colonies respectively.

3.3 Results

3.3.1 Season 2011

Good weather between 23rd and the 29th of August allowed bees to forage on the heather. An average weight gain of 3.04 ± 1.96 kg was recorded and attributed to accumulation of stores (Figure 3.4).

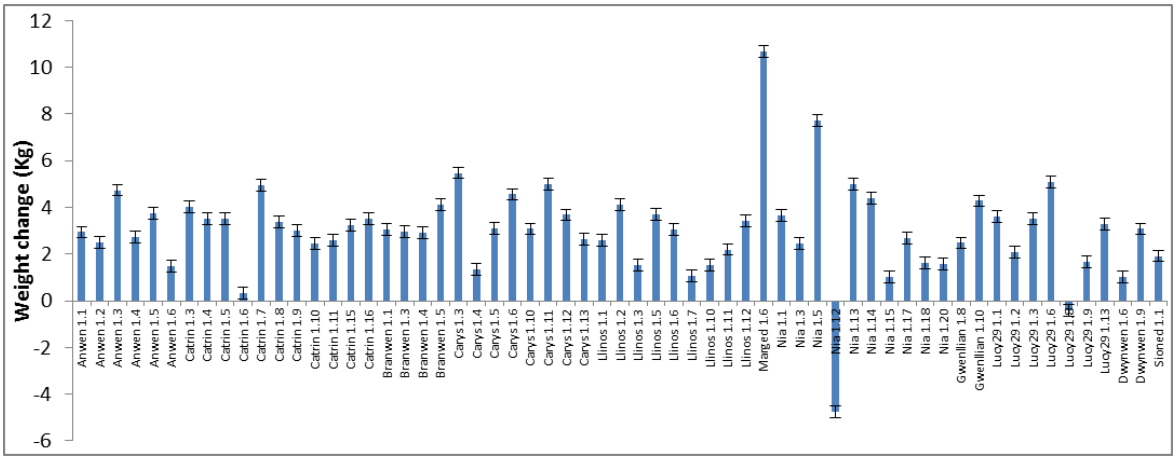


Figure. 3.4 shows changes in hive weights recorded between the 23rd and 29th of August, 2011. Colonies are grouped into breeder daughter cohorts. The error bars indicate one standard deviation of scale precision. This was determined after conducting multiple trials using the scale on a known weight.

The mean number of *varroa* counted, and frames of bees per colony on 1st of October, was 4.90 ± 7.22 and 6.29 ± 1.49 respectively. There was no correlation between weight change during the nectar flow that occurred between the 23rd and 29th of August and colony size at end of season (frames of bees on 1st of October; F= 0.47, R^2= 0.11, P = 0.49), but there was a highly significant correlation between colony weight and frames of bees on the 30th of September (P<0.001; R^2= 0.25; Fig 3.5). There was also a significant correlation between weight change during the nectar flow and weight of colony approximately one month later (P < 0.001, R^2= 0.36). The top 30 colonies were highlighted using a z-score index that accounted for difference in development age (Table 3.3).

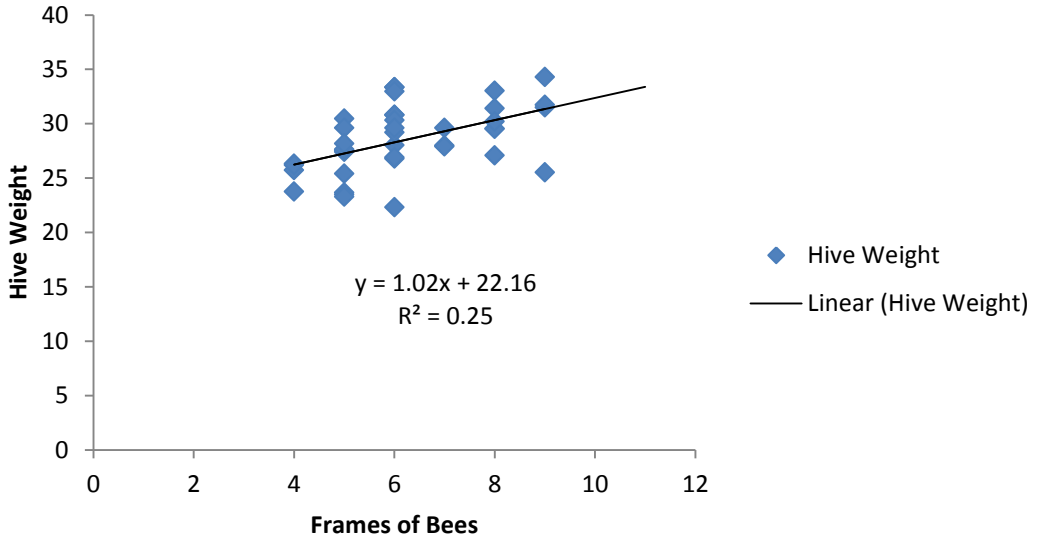


Figure 3.5 The regression of colony weight on frames of bees (colony size) using data collected on the 30th of September and the 1st of October 2011 respectively. Colonies above the linear curve (as predicted by the displayed regression equation) are heavier in relation to the number of bees present, suggesting that bees from these colonies displayed greater foraging efficiency . These better performing colonies are highlighted in Table 3.3, which outlines the top 30 colonies determined using a z-score index.

3.3.2 2012 Season

All successfully mated colonies (N=58) were taken to the heather in 2012. Heather development was delayed due to poor weather and there were no prolonged periods of nectar flow suitable for assessing foraging efficiency. Colonies gained a mean of 2.14 ± 1.81 kg between the 8th and 26th of August, and a mean of 0.87 ± 3.8 kg between 26th of August and the 6th of September. A mean weight change of -0.77 ± 1.31 kg was recorded between the 6th and the 9th of September.

Colonies were monitored for *varroa*, number of frames with bees, and brood coverage, between 28th August and 9th of September. The mean number of *varroa*, and frames of bees counted per colony was 12.18 ± 11.02 and 6.61 ± 0.94 respectively. There was a significant negative correlation between the change in hive weight that occurred between the 6th and 9th of September, and frames of bees and amount of brood recorded during the monitoring period (R^2= -0.19 and -0.36, and P = 0.024 and 0.006 respectively). There was no significant correlation between colony weight on the 8th of September and colony size (frames of bees and brood score; P = 0.09 and 0.09, and R^2 = 0.26 and 0.25 respectively).

Neither was there a significant correlation between weight change and weight of colony one month later ($P = 0.46$, $R^2 = 0.1$). The top 30 colonies were highlighted using z-score index to account for some difference in breeder group (Table 3.4).

Table 3.3. Table of 2011 daughters in order of performance on z-score analysis

Putative Breeders		For Production	
Queen ID	Score	Queen ID	Score
Catrin 1.7	76.61	Anwen 1.6	-4.47
Carys 1.6	45.87	Anwen 1.5	-5.29
Carys 1.5	41.59	Llinos 1.11	-6.75
Catrin 1.3	38.41	Llinos 1.5	-8.90
Catrin 1.9*	31.82	Anwen 1.1	-9.17
Carys 1.3*	30.90	Branwen 1.1	-9.51
Catrin 1.4*	29.59	Anwen 1.4	-11.18
Anwen 1.2*	29.42	Catrin 1.15	-13.77
Lucy29 1.13*	29.18	Lucy29 1.8	-14.33
Marged 1.6	28.72	Nia 1.18**	-15.34
Llinos 1.6	24.30	Nia 1.15	-17.88
Llinos 1.2	24.18	Carys 1.12	-19.54
Lucy29 1.3*	21.52	Branwen 1.3	-20.04
Carys 1.11	17.81	Dwynwen 1.9	-22.33
Lucy29 1.1	15.57	Nia 1.5	-23.12
Catrin 1.5*	15.01	Gwenllian 1.8	-23.31
Catrin 1.8	12.84	Nia 1.14	-25.41
Catrin 1.16	12.60	Lucy29 1.9	-25.92
Carys 1.13*	12.49	Llinos 1.3	-28.02
Anwen 1.3	8.88	Llinos 1.10	-28.44
Sioned 1.1*	5.83	Dwynwen 1.6	-30.44
Llinos 1.12*	5.63	Nia 1.1	-32.31
Catrin 1.6*	0.88	Llinos 1.1	-34.91
Catrin 1.11	-0.45	Nia 1.12	-35.13
Carys 1.10	-0.55	Nia 1.3	-39.73
Lucy29 1.6*	-0.64	Nia 1.13	-46.54
Llinos 1.7	-0.82	Carys 1.4	-52.31
Catrin 1.10	-1.54	Lucy29 1.2***	-56.19
Branwen 1.4*	-2.82	Nia 1.20	-56.43
Branwen 1.5	-3.64	Nia 1.17	-142.10

*Indicates individuals that were above trend line predicted by the regression equation (Figure 3.5)
**Rejected by z-score analysis due to bad temperament
*** Was above trend line but colony heavily infested with mites

Table 3.4. Table of 2012 daughters in order of performance on z-score analysis

Putative Breeders		For Production	
Queen ID	**Score**	**Queen Id**	**Score**
Llinos 2.4	42.57	Catrin 2.9	3.14
Carys 2.8	35.41	Anwen 2.5	1.99
Catrin 2.3	30.44	Anwen 2.6	1.61
Llinos 2.10	30.23	Llinos 2.7	-0.97
Carys 2.2	27.53	Llinos 2.17	-1.80
Carys 2.5	26.10	Carys 2.3	-2.25
Llinos 2.14	25.83	Llinos 2.13	-6.90
Llinos 2.9	24.41	Carys 2.15	-7.18
Carys 2.12	24.20	Carys 2.11	-7.44
Anwen 2.2	21.75	Carys 2.17	-8.46
Carys 2.6	17.47	Carys 2.20	-11.06
Carys 2.4	17.38	Anwen 2.1	-12.97
Anwen 2.4	16.87	Anwen 2.7	-13.72
Llinos 2.6	16.50	Catrin 2.8	-15.37
Anwen 2.9	16.37	Catrin 2.7	-16.24
Carys 2.7	15.85	Llinos 2.15	-21.29
Llinos 2.1	15.64	Anwen 2.8	-22.07
Llinos 2.3	15.17	Llinos 2.19	-23.06
Llinos 2.2	11.80	Catrin 2.5	-23.77
Llinos 2.12	11.73	Carys 2.18	-25.59
Llinos 2.5	11.01	Carys 2.19	-26.10
Catrin 2.4	7.06	Llinos 2.8	-28.48
Catrin 2.2	6.37	Llinos 2.16	-33.63
Carys 2.16	5.80	Llinos 2.11	-48.28
Anwen 2.10	4.98	Carys 2.13	-49.74
Anwen 2.3	4.68	Carys 2.10	-55.74
Catrin 2.6	4.55	Carys 2.1	-82.28
Catrin 2.1	3.92	Carys 2.21	-82.28
Carys 2.14	3.55		
Carys 2.9	3.23		

3.3.3 Testing for difference in variance between years

3.3.3.1 Colony size

Colony strength data (frames of bees and brood) were normally distributed. A one-way analysis of variance (ANOVA) indicated that the mean frames of bees each year were not significantly different ($F = 1.71_{1,93}$, $P = 0.195$). However, Levene's statistic rejected the null hypothesis that variances were equal between years ($F = 10.33$, $P = 0.002$). I therefore re-assed the assertion that mean colony size did not differ between years using a non-parametric approach (Mann-Whiteny). Again, the null of similar means could not be rejected ($Z = -1.73$, $P = 0.08$). Simlarly, Levene's statistic could not reject the null that the variance in amount of brood was equal between years ($F = 12.35$, $P = 0.001$).

3.3.2.2 Varroa

The mean number of *varroa* detected per colony increased drastically and significantly between seasons (Table 3.5), but it was less clear whether population level variance also differed between years. The raw data were positively skewed hence analyses were conducted after log transforming the data. Mean levels of colony infestation were significantly different across years ($F_{1, 87} = 21.57$, $P < 0.001$), and Levene's test could not reject the null hypothesis that variances were equal ($F= 0.312$, $P = 0.58$; Table 3.6).

3.3.4 Temperament

The daughters of one 2011 breeder (Nia) produced colonies of consistently poor temper. Most of these hives scored a 1 (i.e., most unpleasant to work with) on my subjective scoring scale and were very defensive (Appendix i). Quantitative sting tests were conducted on the 2012 daughter colonies, and on a control group consisting of only 2011 queens (Table 3.5). Again, the raw data were not normally distributed (positively skewed) and were log transformation. Analysis of variance indicated no statistical difference in temperament (mean number of stings) between the 2012 colonies and the control group. and Levene's test indicated no difference in variance (Table 3.7). However, a significant difference in propensity to sting was indicated between the two 2012 breeding cohorts (Table 3.7). A significant difference was also detected in the temperament of two of the four daughter groups (Llinos and Carys; Figure 3.6).

Table 3.5 Descriptive statistics comparing colony size, *varroa* infestation and temperament across 2011 and 2012

Frames of Bees	***2011***	***2012***
Mean	6.29	6.61
Standard Deviation	1.49	0.94
Sample Variance	2.21	0.88
Count	38	57
Brood count	2011 (Summer)	2012 (Autumn)
Mean	14.43	11.12
Standard Deviation	4.33	2.94
Sample Variance	18.79	8.65
Count	60	49
Varroa		
Mean	4.90	12.18
Standard Deviation	7.22	11.02
Sample Variance	52.09	121.52
Count	50	39
No Stings		
Mean	11.00	10.75
Sample variance	105.68	170.26
Standard Deviation	10.28	13.05
Count	26.00	57.00

Table 3.6 Comparing means and equality of variance in number of *varroa* detected per colony (2011-2012)

	LnVarroa				t-test for Equality of Means
	Levene's Test for Equality of Variances				
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	0.312	0.578	-4.645	87	0
Equal variances not assumed			-4.594	78.058	0

Table 3.7 Testing for differences in mean colony temperament between years (2011-2012) and between the two 2012 breeding cohorts

	Temperament				
	Levene's Test for Equality of Variances				t-test for Equality of Means
	F	Sig.	t	df	Sig. (2-tailed)
Between years (2011 and 2012)	2.159	0.145	-1.154	80	0.252
Between 2012 cohorts	0.017	0.898	-2.103	53	0.032

Breeder	Anwen	Cartin	Carys	Llinos
Anwen		0.44	3.17	0.11
Catrin	0.51		1.02	1.23
Carys	0.08	0.31		6.23
Llinos	0.74	0.27	***0.01***	

Figure 3.6 Pairwise comparisons for difference in temperament between the four 2012 daughter colony cohorts. The Kruskal-Wallis test statistic is presented above the diagonal and the resulting *P*-value below. Significant difference is indicated by the bold italic number.

3.4 Discussion

Some observable population level shifts were detected after two rounds of breeding. Colonies became more uniformed in appearance, and there was a detectable reduction in the variance of colony size. The mean mite load carried per colony increased significantly during this time, but no significant difference in temperament was indicated between colonies headed by 2011 and 2012 raised queens. There was no detectable difference in the variance of these two colony level traits across years. It is possible that these two traits (mite load and temperament) were influenced by factors not specifically related to the genetics of colony specific queens, and were therefore less influenced by selection and the resulting reduction in genetic variation. Factors such as mite virulence, environment (e.g., weather conditions) and uncertain paternal sources could have influenced the observed expression of these two traits.

Despite the frequently unfavorable weather conditions, each year approximately 75% of the newly established nuclei acquired a laying queen. These results were at par with other sites with more favorable weather conditions (e.g. Shropshire) where the queen cells had been raised by a professional breeder. Selection of low quality larvae (e.g. poorly nourished), or ones that are damaged during the grafting process, can affect the mating success and ultimate quality of the queens, and evidence of possible supersedure was observed in a small number of nuclei in 2012. It is thought that supersedure occasionally occurs in colonies with immature queens since they can have a similar pheromone 'signal' to that of an aging or failing queen (limited brood pheromone in hive). No evidence of inadequate mating as indicated by inferior brood patterns resulting from diploid drone production was observed. Sixty one of 118 colonies were taken to the heather in 2011, and relatively good weather produced nectar flows that allowed the experimental colonies to expand.

There was no correlation between weight change during this flow period, and the estimated strength of the colony. This observation can be explained if there was a large variance in colony foraging efficiency; or in other words, that colonies of similar size tended to accumulate stores at different rates. However, there was a correlation in 2011 between frames of bees and colony weight at end of the heather season. Since increasing production potential is a main project goal, colonies displaying above expected weight gain (in relation to number of bees) were highlighted. Fifteen colonies met these criteria of which thirteen were also highlighted by z-score analysis for further observation as potential breeder stock. Weight gain during nectar flow (rather than weight at end of heather season) was the productivity criteria used in the z-score analysis, so it was reassuring to observe a general agreement between the two methods. The two colonies with above average productivity, but rejected by z-score analysis either had bad temperament (Nia 1.18), or very high *varroa* count (Lucy29 1.2). (see Appendix i). The colony headed by queen Marged 1.6 was also a notable standout. The colony recorded highest net weight-gain during the nectar flow but failed to register above the mean expected weight at the end of the season. Clearly, the production potential of this colony would be missed by relying on colony weight only.

All matured nuclei were transferred to the heather for monitoring in 2012. The inferior quality and short duration of the heather bloom, and the associated prolonged periods of

unsettled weather, provided little opportunity for strong nectar flows and foraging by bees this year. Colonies did manage to expand and steadily gain weight during the first few weeks on the moor, but colony weights started diminishing as the heather flows began to shut down. The rate of weight decline was negatively correlated with the amount of bees in the colony. This makes sense as more bees need more food, and honey stores will deplete at a faster rate in larger colonies. There were no specific periods of strong nectar flows on the heather in 2012; hence no attention was given to foraging efficiency when comparing colonies. Overall weight gained during the whole period when some foraging was possible was therefore used, in relation to colony size, when comparing colonies. Generally though, conditions offered little opportunity to monitor productivity this season.

The propagation of *varroa* tolerance was always a desirable project goal. The need for increasing resistance was highlighted by the heavy losses, attributed to overwhelming *varroa* and associated virus pressures (DWV and parasitic mite syndrome) that were incurred across the winter of 2010/11. Mite counts were taken each year while the hives were on the heather, just as brood production was beginning to slow down and as mite populations approached peak numbers. Although it is known that mite count is only marginally heritable (Harbo and Harris, 1999) counts were taken in an effort to selectively direct the population towards tolerance.

Counts were also conducted to monitor population level infestation rates. Relatively low numbers were detected in the experimental colonies 2011, possibly as a result of the selective sweep the population incurred the previous winter. Numbers increased the following year, (2012) and colonies were treated with an organic acid (3.5% oxalic acid dribble) after brood production had stopped. The situation provided an apparent dilemma; how can one now select for tolerance to local mite parasitism after drastically interfering with host/pest interactions? Commercial operations have limited time and resources to expend on demanding monitoring schedules. Recommendations based on a German approach (Büchler et al. 2010) for evaluating *varroa* mite tolerance in honeybees were recently proposed by the BEE DOC (Bees in Europe and the Decline Of honeybee Colonies) project. The method suggests taking two *varroa* counts (one in the spring and the other later in the summer) to assess mite population growth rates. The process seems well suited for use by well-resourced institutional breeding organizations, and less so by

small scale breeding programs trying to improve overall bee performance by merely selecting from the constantly strongest performing colonies

Bee temperament was unusually bad during the spring of 2012, and although weather conditions were poor and known to influence temper (Villa, 1988), there was concern that the aberrant behaviour was due to breeding. A distinct difference in propensity to sting was demonstrated among the 2012 experimental colonies. However, overall, there was no significant difference between the 2012 experimental population and a control group comprising of 2011 and earlier colonies only. Neither the mean number of stings per colony, nor the variance of the data was statistically different between groups. These assays were conducted after both groups had been migrated to the heather and under very similar weather conditions days apart. These results seem to suggest that environmental conditions may have been the major contributing factor affecting colony temperaments earlier in the year.

However, stinging response is known to have a genetic component (heritability). For example, there are three known stinging behaviour QTL's: *sting-1, sting-2 and sting-3* (Arechavaleta-Velasco, et al., 2003), with *sting-1* being associated with actual stinging response and guarding. Beekeepers commonly try to remedy unmanageable hives by replacing the queen. The new queen mediates the transfer of both maternal (from her) and paternal (from the drones she mated with) *sting* genes into the next generation of diploid workers. It is possible that colony defensiveness can be directly influenced by the colony queen genotype, or indirectly by the haplotypes of the drones she mated with.

Two groups of daughter queens were grafted in the spring of 2012. Each group comprised of daughter colonies raised from the same four breeders, but they were located in different mating apiaries. There was a significant difference in the defensiveness of the two groups when possible maternal effects were ignored. Overall, daughter colonies from the first grafting batch were statistically less defensive than the colonies from the second. Comparing within breeder groups showed that this overall difference was driven mainly by the highly significant difference observed between the two grafting sister-groups of one specific breeder (Llinos). However, a general trend was obvious as there was an almost significant difference between grafting cohort for two of the other three breeders. Numerous environmental variables could have potentially differentially affected the

development and conditioning of the queen larva in these two grafted groups, but it is unknown if such non-inherited influences can affect the temperament of a queens progeny. Mating success is largely environment dependent and there may have differed between groups, but again there is no evidence that a correlation exists between mating success and progeny temperament. Mating success is known to influence queen development and conditioning (Tarpy et al., 2012; Richard et al., 2007) which in turn affects her pheromone induced influence over colony behaviour.

It is also possible (and probably most likely) that difference between grafting cohorts reflects a paternal genetic influence since drone contribution and conditions may have differed. This explanation is very plausible since these two groups mated at different locations occupied by different drone contributing colonies. Different colonies contributed drones to these two groups. Guzman-Novoa et al. (2004) describe the influence of paternal gene transfer on colony level defensive response. They conducted reciprocal cross experiments between honeybee colonies of European and African origin, and observed that hybrid colonies of African paternity were significantly more defensive. They hypothesized that epigenetic influences might be down-regulating (silencing through methylation) major stinging alleles if inherited from the mother in order to reduce the cost associated with having an overly defensive and 'unbalanced' colony. Many drones usually contribute to colony phenotype; hence major defensive alleles will by chance be inherited by a fraction of workers only. Colonies might therefore have a more 'balanced' defensive response if the trait is inherited through the male line. The authors hypothesized a gender specific silencing mechanism and suggest that major defense alleles may not be silenced when inherited from the father.

Assuming the above hypothesis is true; bee-breeders may not be readily able to identify colonies producing drones carrying major defense allele, as these alleles will be silenced in workers when inherited from the queen. But a high percentage of the drones produced by the queen will carry these alleles, and if they successfully mate, their effect will be expressed in the daughters; i.e. the workers in colonies headed the queens they mate with. Colonies headed by queens that mated with multiple drones carrying major defense alleles may become defensive and difficult to manage when.

Although 11 and 4 breeders were selected in 2011 and 2012 respectively, the effective number used each year approximated 8.3 and 3.5 due to unequal breeder representation in the daughter generation. Greater selection pressure was therefore applied during the second round of breeding (2012). There was consequently greater uniformity among the 2012 colonies in both size and organization of the brood nest. This noticeable trend was statistically supported since there was significantly less variance in the numbers of frames of bees occupied by bees at the end of the 2012 season, although there was no difference in means across years. Reduced variance was also noted for brood amount in each nest. Means were also different in this case since comparisons were made using data collected at different times of the year (summer and autumn), and therefore during different colony development periods. However, this trend towards uniformity was notable and suggests that the population as a whole is responding to selection pressure.

One of the goals of the WWBBP is to develop a practical protocol that could help small breeding programs improve the quality of their bees. Broadly, the 'improved' population will trend towards greater productivity, greater disease resistance and gentleness with time. The program has gone through two rounds of selection to date, and there is slim evidence of "improvement". Nevertheless, a trend towards uniformity is clearly indicated by observation and by a statistically significant reduced variance in colony characteristics. The program selected 11 breeders in 2011 and dropped this number to 4 in 2012. Four breeders per generation is the current favored model, a number driven mostly by practical and logistical co considerations, since the program can only accommodate a limited number of new colonies a year (n ~ 80 to 100).

Queen 'rearing' might aid beekeepers to treat the symptoms of poor bee-health (i.e. replacing losses), but it does not necessarily address the root cause of unacceptably high mortality. At worst, an unconsidered approach to rearing could inadvertently exacerbate future overwintering losses and general bee-health (e.g. genetically constrained selection regime). A basic understanding of bee mating biology and the genetic dynamics of bee populations might help avoid such pitfalls. Research has consistently demonstrated a correlation between degree of genetic diversity and general health and fitness at both colony and population level. Genetically depleted colonies or populations have been shown to express reduced vigor and increased susceptibility to disease. Breeders must guard against genetic depletion by rearing queens from unrelated breeders.

Monitoring phenotypic change revealed three key points. First, colony size responded to selection, becoming more uniformed as the population became more genetically constrained. This observation was interpreted as a consequence of effective breeder number dropping to only four in 2012. Secondly, the mean number of *varroa* mites detected per colony increased drastically between years. The program suffered serious *varroa* attributed losses in 2011, hence these results demonstrate the value of monitoring as a tool to manage colony treatment. Lastly, a difference in temperament between the two 2012 breeding cohorts (each comprising of daughters raised from the same four breeders, but mated at different locations) suggest that paternal influences might be affecting overall temperament.

Chapter 4

Selection on Genetics

4.1 Introduction

Line breeding is a common breeding design used by small scale breeders selecting and breeding from within a small closed population (the line). Small operations, such as the West Wales Bee Breeding Program (WWBBP), can generally only resource a single line, within which a limited number of individual queen lines will be maintained. Within-family selection has been recommended when working with relatively small populations such as this (Moritz, 1986). It is considered as the best approach for small scale operators wanting to improve stock quality while concurrently trying to maintain genetic diversity across generations. The basic approach is to each year select and breed from the best performing colony in each breeder family. In conjunction with queen selection, one can also simultaneously manipulate male mediated contributions by using the drones produced by sister queen cohorts that had been raised from strong and vigorous colonies the previous summer. These drones would mediate the transfer of promising grandmother colony characteristics through the male line. This approach has been loosely applied by the WWBBP to date.

4.1.1 Avoiding inbreeding

Charles Darwin (1876) was the first to formally describe the detrimental effects of inbreeding. He demonstrated this by comparing the fitness effects of cross and self-fertilization in numerous plant species. Since then, innumerable studies on both wild and captive populations have demonstrated similar effects in sexually producing organisms. Crnokrak and Roff (1999) subsequently published a significant work suggesting that wild inbred individuals will on average suffer seven times more from the effects of inbreeding depression than similarly inbred captive individuals. Inbreeding depression appeared to be expressed to a greater extent under stressful circumstances. The increased rates of colony losses observed in the Northern hemisphere over recent years indicate that honeybees are experiencing a period of increased stress. It is possible that the multifaceted nature of these challenges could render bees more susceptible to the expression of detrimental inbreeding effects.

Inbreeding is an inevitable consequence of line-breeding (Harbo and Rinderer, 1980) since selection constricts the transfer of genetic material across generations. Inbreeding will eventually be detrimental to breeding efforts since enhancing the expression of desired

characteristics becomes more difficult with each passing generation as selecting for desirable traits will be far less effective in inbred populations. Colonies with inbred bees might also express reduced vigor and may have spotty brood pattern (glossary) due to homozygosity at the sex determination locus. It is commonly argued that the social hymenoptera are particularly susceptible to inbreeding depression due to genetic load on the sex determination locus (*csd)* and to their usually low effective population sizes (Zayed and Packer, 2005) In reality, little is known about the effects of inbreeding in haplodiploid insects (Liautard and Sundström, 2005) and there seems to be limited evidence that it is a problem in large managed commercial beekeeping operations that use open mating (Oldroyd, 2012).

Two hypotheses (dominance and over-dominance) are frequently evoked to explain the expression of inbreeding depression (Zayed, 2009). Firstly, diploid individuals randomly mating in a large population carrying lethal and non-lethal alleles at low frequency will be protected from the deleterious effects of rare maladapted alleles by the masking effect of dominant non-deleterious homologs (dominance). The expression of inbreeding depression becomes more likely in small closed populations due to the increased likelihood that maladaptive alleles become paired due to mating between relatives. In addition, random genetic drift in small populations reduces genetic diversity (since alleles are more likely to be lost in small populations) leading to increased homozygosity and increased likelihood of inbreeding depression (Lande, 1988). The accumulated effect of numerous homozygous loci carrying maladapted genes results in general loss of vigor. This in effect is inbreeding depression. The second hypothesis relating to inbreeding is over-dominance, which suggests that inbreeding is caused by the tendency of homozygotes to have lower overall fitness than heterozygotes.

4.1.2. Genetic variation in honeybee populations

Honeybees have been managed by humans for thousands of years and extensively so in Europe and North America since the middle of the nineteenth century. Domestication in general usually results in loss of genetic diversity (Wright et al., 2005; Zeder et al., 2006) and low levels of genetic diversity have been observed in several European and North American populations (Delaney, et al., 2009; Jaffé et al., 2010; Meixner et al., 2010). In light of the very poor health of many contemporary managed populations (Cobey et al., 2012; vanEngelsdorp and Meixner, 2009) these observations have raised concern that

historical bee management and breeding practices may have resulted in a depleted contemporary genetic pool. Many studies have correlated increased diversity with superior colony robustness and vitality hence the maintenance of variation is important.

However, honeybees are not strictly 'domesticated' and recent work by Harpur, et al. (2012) indicates that managed admixed populations of honeybees in Europe have more genetic diversity than either of their two progenitor populations, i.e., the Western (*M*) and the Eastern (*C*) lineages (Franck et al., 1998; Garnery et al., 1992; Whitfield et al., 2006). Genetic and morphological methods indicate that honeybees spread out of Africa during two separate expansion events and that they were historically geographically isolated into North West and South East Europe. The translocation of bees between these regions was begun by beekeepers and breeders during the middle of the nineteenth century and continues to this day (Meixner et al., 2010). It appears that the constant input of imported stock and the somewhat novel mating biology of honeybees may have allowed diversity to be maintained despite the selection pressures that are applied due to management practices (Harpur et al., 2012; Oldroyd, 2012). There is also evidence from large breeding operations using open mating that neutral genetic diversity is maintained (Oldroyd, 2012).

4.1.3 Effective population size

Population size (N) is a central tenet of evolutionary theory since it has a profound bearing on the response of populations to drift and selection, and on their susceptibility to inbreeding (Waples, 1989). Simple counts or mark recapture methods can provide accurate populations estimates, but the census size can also differ greatly from its effective genetic size (N_e). N_e can be defined as the number of individuals contributing genes to the next generation, but is more equivalent to the number of colonies within effective mating range for honeybees. Consequently, numerous estimators utilizing molecular data from population samples have been developed. These approaches have been used by wildlife managers concerned about the destiny of small populations since they can provide insight into potentially problematic demographic and genetic trends. I employed these methods to investigate changes in population size in a managed honeybee population over a brief contemporary time scale due to selection.

4.1.4 Microsatellite loci and the Complementary Sex Determination (csd) locus

I used a suite of selectively neutral markers and a single marker from a region under strong selection to assess how much genetic variation was in the baseline population. Microsatellite loci are generally assumed to reside in selectively neutral regions of no known function. They are characterized as regions with high mutation rates comprising of two, three, or four base pair repeat sequence motifs bounded by more conserved regions of locus specific sequences. These bordering regions provide primer access for fragment amplification using PCR. Conversely, *csd* is a gene with a described sex-determination function that experiences balancing selection in response to diploid drone production. Bees that are homozygous at the *csd* locus develop into sterile diploid drones that can contribute nothing to the next generation. Low frequency *csd* alleles are therefore favored as they are less likely to match in a homozygous diploid state with zero fitness.

In this chapter I investigate the rate of genetic change occurring in a breeding population under selection across two breeding cycles. The population comprised mainly of bees that had been sourced, over a number of years, from a knowledgeable local bee breeder who had likely taken steps to avoid inbreeding. In light of this knowledge, and of the recent work by Harpur et al. (2012) highlighting the increased genetic diversity found due to translocation in managed bee populations, I considered it likely that high levels of polymorphism would be found at both neutral microsatellite loci, and at the *csd* locus in this population. I also hypothesized that there would be a detectable reduction as a result of selection in both genetic diversity and effective population size (*Ne*) across the monitoring period.

4.2 Methods

4.2.1. Population genetic data sampling

Sampling was designed to investigate the rate of genetic change occurring in a breeding population under selection across two breeding cycles. Samples were taken from the source population (*G0*) which was comprised of colonies that were established in 2010 or earlier. Foraging workers were sampled from the entrance of sixty randomly selected colonies. The 2011 (G1) and 2012 (G2) 'cohort' data were generated using worker samples taken from colonies headed by queens raised during the respective year. The WWBBP selected eight effective breeders from the baseline population to supply queens for the 2011 cohort, and four breeders were selected from this cohort to supply queens for

2012. The daughter colonies of the 2011 (G1) and 2012 (G2) breeders were sampled in a similar fashion to the baseline, with sixty foragers (colonies) sample each year. All samples were stored in 98% ethanol at room temp for preservation and DNA extraction.

4.2.2 DNA extraction

DNA was extracted from equal volumes of leg or thorax tissue using a modified version of the 96 well plate protocol described by Lagisz et al.(2010). The extraction protocol was performed in 1.5ml tubes and the reagent volumes adjusted accordingly. The cell lysis was conducted under moderate agitation at 37°C using a solution comprised of 50mM Tris (pH 8.0), 0.4M NaCl, 0.5% SDS and 20mM EDTA. Lysing was allowed to proceed for two or three days for higher yields. Salt precipitation with 4M Ammonium Acetate was used to precipitate unwanted cell proteins. The salt solution was added to the lysis mixture and centrifuged. The DNA-containing supernatant was poured into anther tube, and precipitated out of solution with ethanol. This tube was centrifuged for 30 minutes to pellet the DNA and the ethanol carefully poured off. After a final wash step using 70% ethanol and a 12-15 min spin, the DNA pellet was left overnight on the bench to dry and rehydrates in 50µl of 1X TE. The extracted DNA was quantified using a NanoDrop nd1000 spectrophotometer and each sample was diluted to 50ng/µl for genotyping.

4.2.3 PCR Multiplex Systems

I designed two multiplex reactions comprising of seven and three microsatellite primer pairs each (Table 4.1). Each multiplex was amplified in a single 10µl multiplexed reaction consisting of 50 ng of DNA, 0.75 X Qiagen multiplex PCR solution, 2.5 and 0.25 pM of reverse and forward primer mixes respectively, and 2.5pM of ABI Fam-, Pet- and Ned- and 5.0pM of Vic-labeled primer. Polymerase chain reactions were performed on a DNA engine Tetrad 2 thermocycler (BIO RAD) using the following cycling parameters: 95°C for 15 min, followed by 13 cycles of 94°C for 45 sec, 55°C for 45 sec and 72°C for 45 sec, and then 25 cycles of 94°C for 45 sec, 52°C for 45 sec, and 72°C for 45 sec. The profile was terminated with a 30 min extension at 60°C. Reaction products were visualized on an ABI 3130xl Genetic Analyzer and the data were analyzed using Genemapper (ABI).

Table 4.1. Microsatellite markers

Locus	*Unified Name*	*Authors*	*Label*	*Accession*
Multiplex 1				
Ap43	*Am098*	*Solignac et al. 2003*	*Pet*	*AJ509329*
A14	*Am406*	*Solignac et al. 2003*	*Vic*	*AJ509239*
A29	*Am014*	*Solignac et al. 2003*	*Vic*	*AJ509245*
A79	*Am046*	*Solignac et al. 2003*	*Fam*	*AJ509277*
A107	*Am056*	*Solignac et al. 2003*	*Fam*	*AJ509287*
A113	*Am059*	*Estoup et al. 1995*	*Ned*	*AJ509290*
Ap14	*Am068*	*Solignac al. 2010*	*Ned*	*AJ509305*
Multiplex 2				
A7	*Am005*	*Estoup et al. 1994*	*Pet*	*AJ509236*
Ac1109	*Am441*	*Solignac et al. 2003*	*Vic*	*AJ 509672.1*
Ap80	*Am124*	*Solignac et al. 2003*	*Fam*	*AJ509355.1*

4.2.4 Csd-marker

The *csd* gene contains a hyper-variable region that is characterized by an arginine-serine rich repeat region, bounded (in a similar way to microsatellites) by more conserved sequence regions. I designed primers (Hypcsd F 5'-CGTTCAAGAGAACGAGAGC-3' and Hypcsd R.1 5'-GTCCCATTGGTCTTGGTGG) to target the conserved regions (Discussed further in Chapter 6) to investigate how variation changes through generation of selective breeding. The primers were designed to generate product fragments of approximately 450 base pairs long to facilitate standardization with the ABI Genescan500 size standard. I attached a tail to the 5' end of the forward primer with a sequence complementary to an ABI Ned labeled tail for fragment visualization. This marker was amplified independently of other markers as I had indifferent success incorporating it into an existing microsatellite multiplex system (i.e. multiplex 1 or 2). The marker was amplified in a single 10µl multiplexed reaction consisting of 50 ng of DNA, 0.75 X Qiagen multiplex PCR solution, 10.0 and 1.0 pM of reverse and forward primer mixes respectively, and 2.5pM of ABI Fam. Same PCR profile was used as for microsatellite markers.

4.3 Statistical Analysis

4.3.1.Overlapping generations

Data analysis and interpretation of results was potentially complicated by the overlapping nature of honeybee generations. Honeybee generations can overlap since virgin queens

can mate with drones produced by much older queens and drone-producing queens can persist for multiple years under natural circumstances. Samples taken from the source population were assumed to be a random sample of a single generation consisting of 2010 and older queens. This sample set was inherently different to the samples subsequent taken from annually produced cohorts groups (G1 and G2). I therefore compared the genetic signature of the source population to each of the cohort years separately, and to the two cohort years combined as a single 'generation'.

4.3.2 Genetic diversity

Genetic diversity has been measured in a number of different ways. Heterozygosity is a very commonly used index. It is the expected probability that an individual carries different alleles (heterozygote) at a single locus, or at an assay of different loci,

$$He = 1 - \frac{1}{m}\sum_{l=1}^{m}\sum_{i=1}^{k} p_i^2$$

where, p_i is the frequency of the i^{th} of k alleles, and m is the number of loci. The observed heterozygosity (H_o) in a population sample is frequently compared to that which would be expected (H_e) under conditions of random mating. Significant deviations will indicate that the population is experiencing an external driver or evolutionary force such as selection or inbreeding. Average heterozygosity is a measure of genetic diversity at the population scale and indicates the average proportion of individuals that are heterozygous for any given trait (locus).

The mean number of alleles per locus (Allelic Richness) is another commonly reported diversity index. It is very sensitive to sample size, and has the disadvantage that information is lost due to rarefaction. Rarefaction is used to determine this index. It allows number of allele estimates from samples of different sizes to be compared, but it does this by scaling from all samples data down to that of the lowest sample size. Nevertheless, the method is useful since it is more sensitive than changes in observed

heterozygosity to recent bottleneck events since it is more sensitive to the loss of low frequency alleles.

Exact tests for Hardy Weinberg Equilibrium (HWE) for each locus and 'population', and genotypic linkage disequilibrium among loci pairs within each population, were computed in GENEPOP (on the web version 4.0.14), and Arlequin (version 3.5.1). Arlequin was also used to investigate genetic structure over all loci between sampling years. FSTAT was used to determine expected and observed heterozygozity and to determine the number of alleles per locus (Allelic Richness) per generation.
Friedman's test (SPSS v.19) was used to test for significant differences in allelic richness and expected heterozygozity across sampling years. Each 'population' is ranked according to its diversity at a particular locus the average rank of each population across all loci is then calculated and the null hypothesis that the ranks do not differ from the expected value is tested using chi-square.

4.3.3 Detecting bottlenecks

The program Bottleneck version 1.2.02 (Cornuet and Luikart, 1996) was also used to try and detect recent reductions in effective population size. Populations that undergo a bottleneck event suffer reductions in allele numbers and a corresponding, but delayed reduction, in observed heterozygozity. That is, the heterozygosity observed immediately post bottleneck will be greater than that expected with the observed allele frequencies (for loci in mutation-drift equilibrium).

Bottleneck runs a "sign test", a "standardized differences test" (Cornuet and Luikart, 1996), and a "Wilcoxon sign-rank test" to test for excess heterozygosity. However, the standardized difference test was not appropriate since a minimum of 20 loci is recommended and it assumes normal distribution of heterozygosity across loci. The Wilcoxon sign test does not assume a normal distribution and tests the hypothesis that the values of HE (expected heterozygosity) from the baseline and post selection cohorts (both separately and combined) were not different (Spencer et al., 2000). The program returns heterozygosity values expected under mutation-drift equilibrium for the Infinite Allele Model (I.A.M), the Stepwise Mutation Model (S.M.M), and the Two Phase Model (T.M.P), and computes if these values are greater or less than would be expected for each model. It provides a *P* -value for each observed heterozygosity. I report results for all

models but place greater emphasis on the TMP since it is known to better model microsatellite evolution (Valdes et al., 1993; Di Rienzo et al., 1994).

The program Bottleneck also produces a "Mode-Shift" analysis (Luikart et al., 1998) as a bottleneck indicator. Here, alleles from all typed loci are grouped into designated allele frequency classes (e.g. 0.1 to 0.9 in Bottleneck, but any class distinction can be used). Since most alleles occur at low frequency in stable populations, an allele distribution histogram creates an L-shaped in such populations. Low frequency alleles are more likely to be lost during a bottleneck, hence a "mode shift" might be observed. There might be fewer low frequency alleles in bottlenecked populations with a greater proportion of alleles occurring at moderate frequency.

4.3.4 Estimating the effective population size (Ne)

The effective population size (N_e) of a haplo-diploid population was described by Wright (1933) as,

$$N_e = \frac{9NfNm}{4Nm+2Nf}$$

where N_f is the number of breeding females and N_m is the number of contributing males. Wright also showed that Ne-haplodiploid = 0.75*Ne-diploid. Diploid workers were sampled for this part of the study, and results were corrected to accommodate haplodiploidy where necessary.

Numerous molecular methods have been developed to investigate changes in population size through time (Wang, 2009). These methods are retrospective in the sense that they use contemporary sample data to construct a hypothetical historical population. Authors have employed a variety of approaches relating to different temporal and special time scales to estimate effective population size (Luikart, et al., 2010). Here, I investigate the effects of artificial selection applied over a very brief contemporary time scale on the effective population size of an experimental bee population. Various genetic signatures (e.g. linkage-disequilibrium, heterozygote excess, sib-ship analyses) are used to infer how populations change in response to chance events, or as in this case, selection pressure. Each breeding generation in the experimental population can be extensively sampled, so observed allele frequency changes should be due mostly to selection and not to random noise introduced by inadequate sampling.

It must be noted that each estimate method assumes that the experimental population approximates a theoretical ideal (the Fisher-Wright population). This 'ideal' population is closed to immigration, has discrete generations and equal sex ratios, experiences random mating, and has non-random variance in reproductive success (Hare et al., 2011). Another important assumption (particularly for sib-ship analyses) is that samples are taken from a single cohort so that analyses are not confounded by misidentified parent offspring relationships (Wang, 2009).

Clearly, there will be non-random variance in reproductive success in the experimental population since only selected breeders will contribute to the next generation. Fifty percent (N = 60) of our breeding population was sampled in 2011, while all (N = 60) colonies were sampled in 2012. Again, any change in frequencies (inferred as changes in N_e) should therefore be due to selection and not drift due to sampling chance. Directional selection due to commercial fishing has been recognized in wild fish populations. Temporal shifts in allele frequencies indicated a reduction in N_e which likely caused by reduced variance in reproductive success (Hare et al., 2011).

Honeybee populations also deviate from the 'ideal' model in two other obvious ways. Firstly, laying queens can persist for more than one year under natural circumstances, so that generations can overlap. Most N_e estimators are designed to generate discrete generation estimates, but will provide a related parameter, $N_{b,}$ when samples are taken from a single cohort of a population with overlapping generations. The parameter, $N_{b,}$ is the effective number of breeders contributing to that year (Hare et al., 2011), and is such that $N_e > N_b$ = generation time * $N_{b.}$ My baseline samples were selected from all the colonies that were raised 2010 or earlier, and should represent a random sample of the 'complete' pre-selection generation. Contrastingly, the 2011 and 2012 cohorts were sampled from same-aged queens raised that year. Single sample cohort analyses should therefore provide an indication of the number of contributing breeders (N_b) and should be less than the effective size of the whole population.

Secondly, the haplodiploid nature of bees and the polyandrous nature of the queen further complicate the situation since they result in unequal sex ratios (many more drones than queens). The Colony program accommodates haplo-diploidy, otherwise results need to be weighted by a factor of 0.75. {i.e. N_e-haplodiploid = 0.75* N_e-diploid}.

Finally, the relatively short nature of this study limits the precision of the two samples for estimating N_e methods described below. For precision, it is recommended that a sample-span of two or more generations be used with these methods. I include a discussion and report results on their use below of completeness, but will concentrate single sample results which generally report N_e on the previous generation.

4.3.4a Estimating N_e using single sample approaches

These methods are appealing since only one sample from the monitored population is required. One does not have to wait for generations and a second round of sampling. They estimate N_e by using genetic signatures observed in the one sample data using patterns related to various genetic parameters including linkage among alleles from different loci, heterozygosity and patterns of relatedness among sampled individuals. Recent developments have benefited from recent advances in computing capacity and have shown promising results with these approaches (Hare et al., 2011). For example, Waples and Cho (2008) recently published LDNE which has a bias correction for estimates of (N_e) based on linkage disequilibrium data, and Wang (2009) developed the sib-ship assignment (SA) method for estimating N_e from single generation samples. The method is implemented in the program Colony (Jones and Wang, 2010) and relies on the fact that individuals from the same cohort are more likely to be related (as-sibs) in small populations. Individuals are more likely to share a common parent or parents when the parental cohort is small. All N_e estimates make assumptions about the sampling protocols and populations. The SA method is most sensitive to deviations from single cohort sampling since individuals from different cohorts could be parent-offspring and mistakenly assigned as sibs. Such false assignments would mistakenly lower N_e.

4.3.4b Estimating Ne using temporally based methods

Temporally based methods utilize changes in allele frequencies across generations and hence require at least two different temporal samples from the population of interest. The methods work best when the degree of change due to drift or (as in our case) selection is large since the disruptive effects of changes due to random drift are drowned out of the "signal". These methods should therefore be applicable to a selective breeding situation since we should be imposing enough selection to impose an adaptive response from out population. We can also accommodate large and very accessible sample numbers (large numbers of bees in a colony) in relation to relatively small population sizes. Returned

values should therefore indicate changes due to selection as opposed to random drift due to sampling effect. Additionally, sampling more loci enhances precision (Waples, 1989).

4.3.5 Moment-based temporal methods

Moment-based methods utilize the relationship between Wright's F statistic (1951) and genetic drift. One formulation of F utilizes the expected variance in allele frequency (p_t) between time 0 and t, when adjusted for its starting frequency [p_0 (1- p_0)], and is given as,

$$\frac{Var(p_t)}{p_0(1-p_0)} = \frac{E(p_t-p_0)^2}{p_0(1-p_0)} \sim \frac{t}{2N_e}$$

when ($\frac{t}{2N_e}$) is less than 0.15 (Nei and Tajima, 1981). The expectation of F can therefore be estimated from the observed variance in allele frequencies across samples since this result can be converted to give an estimate of N_e.

It is thought that these methods tend to upwardly bias the estimator when low frequency alleles are encountered. Highly variable microsatellite markers are susceptible to this source of bias since they might carry numerous alleles at low frequency. Bias is also introduced if drift (or selection in our case) is strong enough to result in loss of alleles between samples (Waples, 1989). Precision improves and bias due to overlapping generations decreases with increasing number of breeding cycles between sampling (Waples and Yokota, 2007).

4.3.5a Coalescent based temporal method (TM3)

I also used the program TM3 (Berthier et al., 2002). This approach applies the coalescent model in a temporal method framework. The model is based on the higher expected rates of coalescence when historical populations are small. The convers will also apply, and lower rates of coalescence should occur between the recent and historic samples when historic populations are large. Coalescence based estimators can more readily accommodates continuously reproducing (overlapping generations) populations rather than models based on discrete-generation Wright-Fisher populations (Anderson, 2005). I ran this method multiple times to ensure consistent results.

4.4 Results

4.4.1 Microsatellites (neutral markers)

Some alleles at locus A29 could not be confidently resolved due to the nature of the marker signature and this locus was dropped for the analysis reported here. In addition, pairwise comparisons for linkage disequilibrium suggested a non-random association between alleles at locus A7 and A14. Significant test-results (P<0.002) were observed across all three sampling years. Locus A14 was removed from population structure analyses. No significant deviations from expected Hardy-Weinberg proportions were detected at any locus in any sampling group (χ^2 (60) = 86.68, p =0.014> 0.006 after Bonferonni corrections). No deviations from Hardy-Weinberg expectations (HWE) was observed when the 2011 (G1) and the 2012 (G2) cohort groups were combined (χ^2 (16) = 15.91, p =0.328).

The total number of alleles sampled at each locus across the sampling period ranged from 7 (A441) to 26 (A107). In the baseline population (G0), the number of alleles per locus (allelic richness) ranged between 6.7 (A441) and 22.9 (A107). In the 2011 (G1) and 2012 (G2) populations, the numbers of alleles per locus ranged from 5.0 and 4.0 (A441) to 17.9 and 18.3 (A107) respectively (Table 4.2).

The mean expected heterozygosity (all loci) for G0, G1 and G2 were 0.786, 0.789 and 0.777, and the mean observed heterozygosity values were 0.783, 0.800 and 0.798 respectively (Table 4.3).

Table 4.2 Allelic Richness

Locus	Go	G1	G2
A7	9.60	9.61	7.99
Ap43	9.60	8.88	7.87
A14	12.22	11.70	13.64
A29	21.37	18.97	21.14
A441	6.74	5.00	4.00
A79	11.49	9.61	9.85
A107	22.94	17.93	18.33
A113	10.58	7.85	8.73
Ap14	9.84	9.7	7.98
CSD	31.82	27.71	24.57
Mean	14.62	12.69	12.41

Friedman's test for repeated measures revealed a statistically significant difference in allelic richness, χ^2 (2) = 8.00, p = 0.018. Post-hoc analyses using Wilcoxon-signed-rank tests (SPSSv 19) was therefore conducted, and Bonferroni corrections were applied to correct the significance level for multiple tests (p < 0.05/3). Allelic richness in the 2011 and 2012 samples were both significantly different to the baseline (2010) (Z = -2.20, p = 0.028, and Z = -2.37, p = 0.018 respectively). A significant difference in allelic richness

was also detected between the source population and the two cohort sample groups combined (Z = -2.028, P = 0.022 1-tailed). There was no significant difference in allelic richness between the 2011 and 2012 samples (Z= -1.352, p = 0.176) and no statistically significant difference in expected heterozygosity (χ^2 (2) = 1.143, p = 0.565) between any of the sampling periods.

4.4.2 Complementary sex determination (csd)

A total of 44 different fragment lengths were detected in the hyper-variable region of the *csd* gene. These fragments ranged in size from 407 to 493 base pairs long, with 33, 28 and 25 'alleles' detected in G0, G1 and G2 respectively. There was a consistent decline in number of alleles detected each year at this locus, and a corresponding drop in allelic richness at this locus across the sampling period (Table 4.2). Since this locus experiences strong selection, and will behave differently to microsatellites in evolutionary terms, it was removed from further analysis.

Table 4.3. Expected and observed heterozygosities at all markers

Locus	G0	G1	G2
Expected			
A7	0.73	0.79	0.79
Ap43	0.79	0.84	0.82
A14	0.80	0.82	0.86
A29	0.90	0.91	0.90
A441	0.65	0.54	0.56
A79	0.80	0.79	0.79
A107	0.93	0.92	0.91
A113	0.77	0.81	0.75
Ap14	0.79	0.80	0.7
CSD	0.93	0.94	0.94
Mean	0.81	0.82	0.81
Observed			
A7	0.72	0.72	0.84
Ap43	0.85	0.88	0.78
A14	0.78	0.84	0.85
A29	0.86	0.92	0.87
A441	0.60	0.62	0.62
A79	0.81	0.81	0.83
A107	0.87	0.92	0.89
A113	0.76	0.72	0.74
Ap14	0.84	0.89	0.85
CSD	0.86	0.92	0.90
Mean	0.79	0.83	0.82

4.4.3. Bottleneck

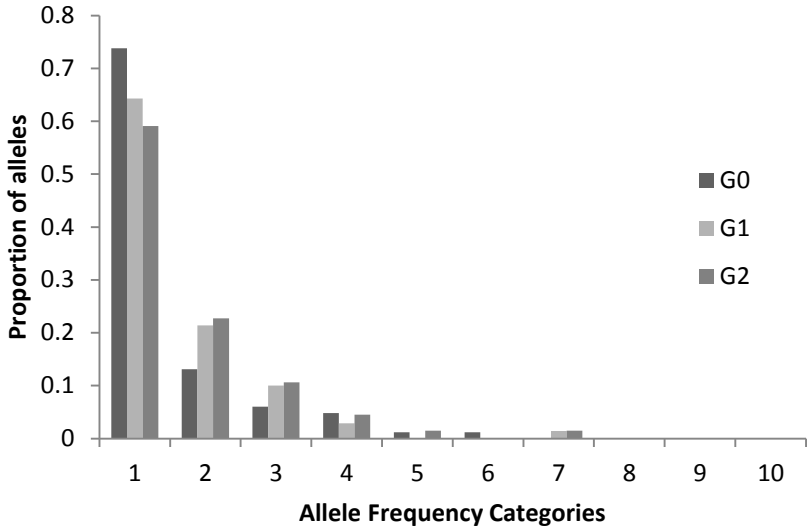
All sample groups had significant heterozygote excess under the IAM. Significant *P*-values were also returned by the Wilcoxon-signed-rank test for the 2011 and 2012 samples under the T.M.P model and by the sign rank tests in the 2012 samples under S.M.M (Table 4.4a). Similarly, Bottleneck detected significant heterozygote excess when both cohort years were combined as one. Again, the Wilcoxon signed rank test indicated significant heterozygote excess under the T.M.P model (Table 4.4b). Normal L-shaped distributions were described for each sampling year's allele frequency distribution (Fig. 4.1a) and when cohort groups were combined (Fig 4.1b). Nevertheless, a progressive shift towards alleles of moderate frequency was observed across years, with alleles of low frequency becoming less prevalent in each successive sampling year.

Table 4.4. Testing for excess heterozygosity with Bottleneck

		Test	IAM	TMP*	**SMM**
a)	G0	*Sign*	***0.028***	0.295	***0.002***
		Wilcoxon one tail for HE	***0.004***	0.961	1.000
	G1	*Sign*	***0.028***	0.415	0.312
		Wilcoxon one tail for HE	***0.004***	***0.039***	0.961
	G2	*Sign*	***0.026***	0.148	***0.021***
		Wilcoxon one tail for HE	***0.004***	***0.012***	0.973
b)	G0	*Sign*	**0.031**	0.103	**0.002**
		Wilcoxon one tailed for HE	***0.004***	0.961	1.000
	G1+G2	*Sign*	***0.023***	0.152	**0.022**
		Wilcoxon one tailed for HE	***0.004***	***0.019***	0.992

IAM Infinite Allele Model
*The TMP (model is the most appropriate for use with microsatellites SMM Stepwise Mutation Model
HE Heterozygote Excess
Bold and italicised p-values are significant

a)



b)

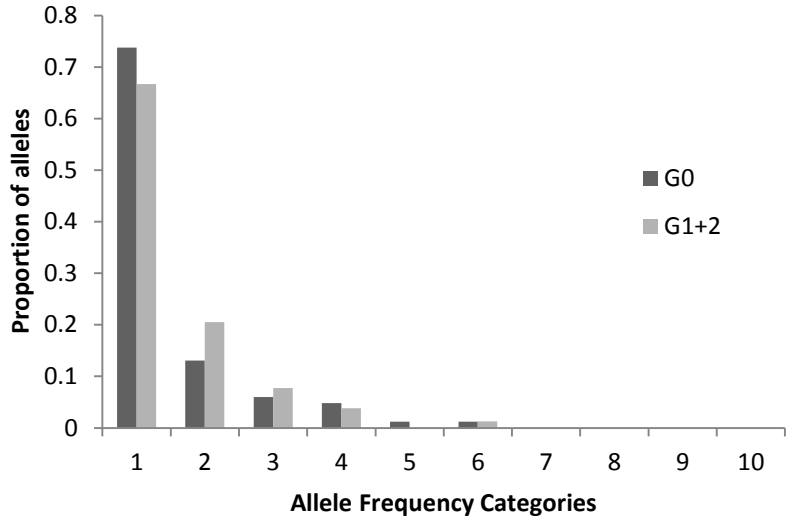


Figure 4.1 The program Bottleneck designates 10 allele frequency categories. This means that the proportion of the total number of alleles across all loci that occur at frequencies < 0.1 are indicated in category 1. Similarly, the proportion of the total number of alleles across all loci that occur at frequencies ≥ 0.1 and < 0.2 are indicated in category 2 etc. Most alleles will occur at low frequencies (category 1; <0.1), but a shift towards higher frequency categories (category 2 and 3) is observed each generation when low frequency alleles are lost due to selection. Such 'mode shifts' are observed in bottlenecked populations. Similar results were observed when generations G1 and G2 were considered independent (a) or when combined (b).

Table 4.5. Ne estimates generated by two single sample and two temporal method estimators using diploid co-dominant markers

Method	Program	Ne - G0	Nb - G1	Nb - G2	Ne - (G1+2)
a) Single sample[¥]	LDNE (d)	**60** (*38-115*)	**46** (*31-77*)	**36.1** (*25.2 - 55.5*)	**55.1** (*41.0 - 76.6*)
	LDNE (hd)	**45** (*28.5-86.3*)	**34.5** (*23.3- 57.8*)	**27.1** (*18.9 -41.6*)	**41.3** (*30.8 -57.5*)
	COLONY*	**46** (*30-72*)	**38** (*24-61*)	**26** (*12 - 34*)	**46** (*23 - 51*)
		Ne - (G0-G2)	**Nb - (G0-G1)**	**Nb - (G1- G2)**	**Ne - (G0 - G 1+2)**
b) Temporal[€]	TM3 (d)	**68.1** (*34.7-100*)	**37.9** (*16.8-71.3*)	**29.7** (*16.7 - 52.1*)	**38.9** (*19.6 - 82.9*)
	TM3 (hd)	**51.1** (*26.0 - 75.0*)	**28.4** (12.6 - 53.5)	**22.3** *(12.5 - 39.1)*	**29.2** (*14.7 - 62.2*)
	Moments Based (d)	**88.9** (*43.3-259*)	**39.4** (*20.0 - 100.4*)	**32.2** (*16.7 - 81.5*)	**52.4** (*26.9 - 130*)
	Moments Based (hd)	**66.7** (*32.5 - 194*)	**29.6** (*15 - 75.3*)	**24.2** (*13.3 - 61.1*)	**39.3** (*20.1 - 98*)

* COLONY readily accommodates haplodiploid data. Other estimators assume diploidy (d), hence the estimate is adjusted as N*e*-hapoldiploid (hd) = 0.75 *N*e*-diploid (d). The numbers in brackets indicate 95% confidence intervals.

[¥] Single sample estimations of number of contributing individuals (colonies) from the previous generation N*e* or previous year N*b*

[€] Temporal estimators provide a mean population size between samples G0 = source population, G1 = 2011 daughter colonies, G2 = 2012 daughter colonies and G1+2 = 2011 and 2012 daughter colonies combined.

4.4.4 Assessing Effective Population Size (N_e)

4.4.4a Single sample methods

The program Colony can accommodate single sample diploid (worker) data within a haplo-diploid context and returned $N_e = 46$ (Table 4.5a) for my baseline (assumed to be a random sample of a single generation) samples. LDNE accommodates single sample diploid data and returned $N_{e.d\ (diploid)} = 60$ or $N_{e.hd\ (haplodiploid)} = 45$ [*as* $N_{e.hd} = 0.75\ N_{e.d}$] for this baseline sample data set. Colony returned N_e's of 38 and 26 for the 2011 and 2012 cohort's respectively, while LDNE returned N_e 's of 34.5 and 27.1 for these data after correcting for haplodiploidy (Table 4.5a). Finally, N_e of 46 and 41.3 were determined by Colony and LDNE respectively after pooling the 2011 and 2012 data as one 'generation'.

4.4.4b Two sample temporal methods

TM3 returned values of 28.4, 22.3 and 29.2 for sample sets (G0 - G1), (G1- G2), and [G0 – (G1+ G2)] respectively when corrected for haplo-diploidy. Similarly, the same data combinations using the Moments Based approach gave N_e's of 29.6, 24.2 and 39.3 respectively (Table 4.5b). Two sample analyses with the programs TM3 and Moments Based of the baseline (G0) and 2011 (G1) cohort data retuned values of 37.9 and 39.4. Values of 29.7 and 33.2 were returned when the using the 2011 and 2012 sample datasets.

4.5 Discussion

The test population was genetically diverse. Most of the microsatellite loci were highly polymorphic, and the *csd* locus was extremely so. These results were not unexpected, and I hypothesize that much of this observed diversity originates from the sourcing stock which is a managed stock of likely mixed genetic heritage. In addition, monitoring demonstrated how low frequency alleles were lost across generations, and allelic richness decreased significantly due to the selection pressure applied. There was also relative congruence among a suite of estimators indicating (as expected) that the effective population size was decreasing as population level diversity dropped.

Although these data suggest that ample variation is present within this population, broader genome-wide variation is not necessarily inferred since correlations between phenotypic variation and variation observed at a small number microsatellite markers are generally weak (Coltman and Slate, 2003). Indeed, these authors suggest that many markers of this type (~600) are needed to powerfully detect inbreeding on life history traits.

Nevertheless, my observations were encouraging, as it appears that historical management practices have not diminished variation and adaptive potential in this population. Indeed,

the opposite might apply as it is now assumed that managed bee populations might carry higher levels of genetic diversity than previously thought This new paradigm is based on research showing that admixture between the two post-expansion (out of Africa) progenitor lineages in Europe has enhanced the overall diversity of this group (Harpur et al., 2012). Although my investigation of genome level variation was not extensive, I see no lack of diversity in this managed population. This conclusion is supported by sequencing data of putative *csd* variation (chapter 6).

The WWBBP breeding model developed in part around available resources, and was tailored to fit into an already established busy commercial beekeeping season. Since the program suffered severe losses over the 2010/11 winter, approximately 110 replacement colonies were raised. Approximately 8 effective breeders were selected from the surviving colonies to contribute to this cohort. Only 60 replacement colonies were successfully raised in 2012, and queens were raised from only four breeders (effective number was 3.5) selected from the 2011 survivors. The drone producing colonies were derived from the remainder of the previous year's queens, and from older breeder colonies. Drones from field apiaries might also contribute as no effort is made to remove then when mating nucleus colonies (glossary) are being constructed in the field. Drones from production colonies are brought back to the mating apiary where they could mate with the test queens. What genetic effect could monitoring detect to date, and what clues might these results provide concerning the long term sustainability of this population if the breeding strategy was maintained?

There was an obvious and significant decline in allelic richness (loss of diversity) at both marker types following the first round of selection. There was a significant difference between the baseline source 'generation' and the first cohort (2011) group in the mean number of alleles per locus detected. Similar comparisons between the baseline and the 2012 cohort demonstrated significant difference in allelic richness, but no significant difference was indicated between 2011 and 2012.

Allelic richness was also significantly lower when the data from the two cohorts were combined and compared to the source. These data seems to indicate that the initial round of breeder selection reduced allelic richness in the 2nd generation. Honeybees have overlapping generations and combining the separate cohort data might better represent a single generation frequency distribution. The resulting dataset should therefore provide a more compatible sample for comparison with the source population which was assumed to be a random sample of 2010 and older queens.

Analysis of heterozygote excess (HE) in the source population and two cohort groups with Bottleneck support this interpretation since significant HE was indicated in both the post-selection cohort groups, but not in the source (2010 and older queens) under the two phase model (T.M.P), the most appropriate model for microsatellite evolutions used in the program Bottleneck. This trend was also observed when the cohort groups were combined. These results are consistent and indicate that a reduction in effective population size has likely occurred due an imposed bottleneck (selection) event.

I explored the influence of selective breeding on the effective size (N_e) of this managed honeybee population. Selective breeding potentially limits the transfer of genetic material across generations, and might therefore result in a reduction in N_e with time. Small populations also tend to lose genetic diversity more rapidly than population of larger effective size so that rate of genetic depletion might also accelerate with time. The combined effects of selection and ever increasing pressures due to reduction in population size might be of concern in the long term.

There was general congruence between the estimates provided by the programs LDNE and Colony with both methods indicating a gradual but consistent reduction in population size with each round of breeding. Single sample estimates such as these generally report the estimated population size of the previous generation (Hare, 2011), but interpretation of the results in this case is complicated by the differing composition of the samples. The source population (G0) was a random sample from a multi-age structured population, while the G1 and G2 cohorts comprised of daughter queens raised selected set of breeders. I tried to address this potential issue by combining the G1 (2011) and G2 (2012) daughter cohort data into a single 'generation', which I considered to be two years in this case. A reduction in Ne was observed using LDNE but not with the program Colony when the source population was compared to the cohort years combined, although the 95% CI was smaller in the latter group in both cases. It is also possible that I am overcomplicating this issue, and that for simplicity's sake, samples could readily be considered as separate generations. Although drones from a small number of productive established colonies had access to cohort queens each year, many of the drone producing colonies were produced the same time as the breeder colonies now providing the next generation of queens. Incoming drones picked up during nucleus colony making will likely introduce most uncertainty into the equation

I therefore also considered G1 and G2 separately as single cohorts, and used LDNE and Colony to estimate the effective number of parents contributing to each N_b. Four breeders

were used to parent G2, so that a minimum N_b of 32 might be expected if each mated with 7 drones (4 queens + 28 drones = 32). This number compares favorably with the single sample estimates of 27 and 26 determined by LDNE and Colony respectively. Twice as many breeders contributed to G1, hence the number of contributing parents should be twice as much as G1 (N = 64). Both N_b estimate generated by LDNE and Colony (34.5 and 38 respectively) were lower than expected.

Temporal method estimates must be interpreted differently, and provide instead information that is relevant across the temporal period. They also tend to work better for populations with discrete generations (Wang, 2005). Probabilistic methods have been shown to have higher accuracy and precision that moments based approaches (Berthier et al., 2002; Tallmon et al., 2004) which tend to overestimate N_e when genetic drift is strong (or selection in this case) and when markers with high allelic diversity are used (Wang, 2005). These limitations might explain the higher estimate values generated by the moment based approach in this study. These methods display greater precision with increasing temporal separation between samples, and might be handicapped here since there is not much generational separation between samples. While the accuracy of the estimates might therefore be questionable, a general decline in population size was again observed.

No concrete inferences can be made based on these results, partly because the unusual nature of honeybee genetics complicates the interpretation of N_e estimates for both simple sample and temporal approaches. Nevertheless, a consistent trend across all methods is evident. It seems plausible to suggest, particularly if one ignores the potential influence of overlapping generations, that based on all the genetic evidence presented here, diversity was eroded across generations and that the effective population size was trending down with each round of selection.

Genetic monitoring revealed two main points. First, the source population displayed high levels of genetic diversity at microsatellite loci, and at the hyper-variable region of the *csd* locus. From a practical perspective, this diversity suggests that the population might hold adaptive potential, that can be targeted by selection Secondly, the current selection regime seems to be an eroding force on this diversity. Results indicate that low frequency alleles are being lost, and the effective population size is diminishing, but it is not clear if the current rate of genetic depletion significantly dampens the adaptive potential of this population. In the next chapter, I investigated the potential short term consequences of this selection regime by modelling changes across five generations.

Chapter 5

Monte Carlo simulation-modelling the influence of various breeding parameters

5.1 Introduction

Predicting the genetic (and hence in part the phenotypic) consequences of selective breeding in a population of honeybees is complicated by the nature of haplodiploid genetics, and by the multiple mating behaviour of queens. In order to better understand the processes involved, honeybee researchers have used computer simulations to model different closed population breeding scenarios (Page and Laidlaw, 1982a, 1982b; Page and Marks, 1982; Moritz, 1984, 1986; Omholt and Ådnöy, 1996; Gupta et al., 2012). Page and Marks (1982) and Page and Laidlaw (1982a, 1982b) were the first to use computer simulation models to investigate the effects of random mating in a closed haplodiploid population. They specifically investigated the effects of inbreeding and drift on the population genetics of sex alleles in genetically isolated artificial populations that were maintained by instrumental insemination (II). By altering their selection criteria and population sizes, they used their simulation model to estimate the rate of decay in brood viability due loss of sex alleles over 40 generations.

Selection and inbreeding not only affects the sex locus, but will also reduce fitness due to inbreeding depression. Moritz (1984) developed a mathematical model relating inbreeding depression (quantified as an inbreeding coefficient) to population size, and illustrated how inbreeding effects depend upon the number of queens selected each year, and on the number of generations since selection started. This theoretical study also estimated genetic progress by using published data from 'real' populations, and illustrates how different maximum improvement limits exist for populations of different sizes. Smaller populations will have lower maximum improvement limits (reduced adaptive potential), and take fewer generations of selection to reach them.

Simulations have also been used to compare the effects of different selection methods. Moritz (1986) and Omholt and Ådnöy (1996) compared within-family selection (selecting the daughter queen with the highest phenotypic value for each breeder queen) and mass selection (selecting the new breeder queens ignoring familial relationships designs), and concluded that under most circumstances, mass selection provides the greatest improvement, although it may be wise to select from within families when population size is small, and when inbreeding is more of a concern (Moritz, 1986).

A universal assumption made by these closed population simulations, and one that may not apply in practice (discussed below), is that each generation of test daughter queens mate only with drones produced by the same group of breeder mothers, and that the semen from all the selected breeder drones can be pooled and homogenized before being

used to inseminate the test queen population. In this way, the models simulate population situations where Instrumental Insemination (II) is used to maintain isolation. They also run simulations for up to forty generations with earlier studies using relatively low numbers of iterations (20-100). Few breeding programs last this long, so that information on contemporary scale is lacking. All these simulations generally assume relatively large population sizes, with number of breeders selected each generation ranging from 10-50, though Moritz (1986) did run simulations comparing a range of breeder numbers ranging from a low of 1 to a high of 50, and mathematically modelled the effects of inbreeding on genetic improvement over 40 generations selecting 2-19 breeders each generation. Maximum character improvement was much lower and is reached more quickly when lower breeders numbers are used.

5.1.1 My model designs

These models were designed to provide practical guidance for small scale breeding programs wanting to improve stock quality through open mating and adaptive selection. The basic model structure assumes a single closed population from which a determined number of breeder queens are selected each generation. A specified number of daughter colonies are then raised from these breeders each year and these in turn provide breeders and drones for the next generation. The methodology here deviates from the closed population modelling structure applied by earlier research (described above), and does so to better simulate the approach adopted by the West Wales Bee Breeding Program (WWBBP). Here, drones from all daughter colonies raised the previous year can contribute to the next generation.

I developed simulation models in MATLAB, using a Monte Carlo sampling approach (codes in Appendix iii and iv). The Monte Carlo method was originally conceived by Stan Ulam in 1943, and became widely applied in later years with the advent of computing technology (Eckhardt, 1987). Monte Carlo simulations can accommodate multiple variables and repeatedly sample probability distributions to come up with many possible answers. When repeated frequently enough, the results can provide a level of confidence or uncertainty about the possible real outcome of the model. I use total allele frequency variance per locus (sum of the variance of all alleles at a single locus) as a model indicator to test for differences between parameter variables.

I developed two models to track genetic change due to selection in a closed haplo-diploid population. The first model simulates population level changes in allele frequencies at

selectively neutral co-dominant loci (one locus at a time). It can accommodate up to 12 alleles of specified frequencies, assumes a closed population, and includes a suite of variable parameters: number of breeders selected each year, number of daughter queens raised, and mean number of drones that each of these daughter queens mates with. To accommodate the overlapping nature of honeybee generations (in this managed situation), the model also assumes that these daughter queens mate randomly with drones from the previous year's colonies. The model runs for 5 breeding cycles and simulates changes due to breeding on a contemporary scale. Simulated results were compared with observed allele frequency distributions as determined through genetic monitoring. One of the aims of modelling was to investigate the relative influence of various breeding parameters (both within the control of, and independent of breeder intervention) on the rate of allele frequency shifts and genetic depletion, for this specific small scale breeding approach. Logic dictates that population level diversity will be constricted if only a selected set of individuals (males and females) taken from the population (of a certain size) is allowed to reproduce each year. Parameters were adjusted in an effort to optimize the outcome. From an applied beekeeping and breeding perspective, this means optimizing the input of effort (the amount of time, effort and money required to select breeders and raise new colonies) for maximum output gain (maintaining adaptive diversity).

The second simulation examined the influence of selection parameters on the maintenance of *csd* diversity within a closed breeding population. Unlike selectively neutral microsatellites loci, *csd* experiences balancing selection as alleles of low frequency are preferred due to a lower probability of being matched (in a homozygote state) by chance in diploids (Charlesworth, 2004). Homozygotes were therefore continually purged from this simulation; otherwise it was similar in principal and construction to the neutral marker model. It models a closed population, assumes random mating, and drone alleles are generated by queens from the previous year. Input parameters included: number of new daughter colonies (queens) started per year, and mean number of contributing drones per queen. The final version accommodates up to 11 alleles of designated frequency, and the number of breeders selected for each separate round of breeding can be individually set. In addition, for each breeding cycle, I calculated the probability that alleles identical by descent would match at random in a diploid individual. For a specific sex allele, this probability was assumed to equal the product of its frequency in the randomly selected breeder pool, and its frequency in the drone producer colonies from the previous

generation. The 'probability of homozygosity' was then simply converted into expected mean population brood viability as follows:

% brood viability = 1 – (probability of homozygosity)

The reciprocal of the probability of homozygosity (or 1- brood viability at *csd*) is equal to the effective number of alleles in the population (Yokoyama and Nei, 1979). The brood viability output provided by simulation was easily converted to provide an indication of change in effective number of sex alleles through time. These three population level parameters are related as homozygosity becomes more likely, and mean brood viability decreases, if the number of sex alleles carried becomes diminished due chance or selection.

A number variables were modelled in these simulations (e.g. number of breeder queens, population size, and number of drones each daughter queen mates with). Of these, I expect the number of queens used/selected each year to have the greatest bearing on genetic preservation across generations. In addition, genetic monitoring had indicated that the WWBBP population was losing diversity under the current breeding protocol. I modelled the WWBBP's current protocol, and expected significant increase (indicating loss of genetic diversity) in allele frequency variances across generations at both neutral markers and at the *csd*. In the latter case, I also expected modelling to illustrate that mean brood viability per colony could not be maintained above 85% in the long term using only four breeders per year.

5.2 Methods

5.2.1 Microsatellite methodology

Observed source population data gathered at four microsatellite markers (A7, A79, A441, and Ap43) were entered into the neutral model (Table 5.1). Initial runs simulated the breeding program protocols that the WWBBP used during the first two selection cycles; i.e., eight breeders were selected at random from a simulated source population (G0), and four were selected from the resulting generation (G1). One hundred new queens were raised each year, and each was assumed to have mated with seven drones. I initially ran each simulation between 1000 and 5000 times. The higher number of model iterations took very much longer to run and no significant advantage in precision (difference in standard deviations) of the results was gained. Simulations were therefore run 1500 times

and I chose 1 standard deviation as a measure of model predictive precision. That is, I assessed whether the observed allele specific data acquired through genotyping a population sample were within one standard deviation of the simulated mean.

I then adjusted the model breeding parameters to independently assess their influence on the genetic stability of the population through five simulated selection cycles. For each locus, a frequency variance was calculated for each simulated allele frequency distribution, and these were then totalled to give total variance per locus. I initially compared total variance when 4 and 30 breeders were selected, and when 100 or 1000 colonies were raised each year

As there is evidence suggesting inferior mating success in this region, I also adjusted the mean mating success of queens in the population with all other parameters fixed and replicating the WWBBP protocols. The mean number of drones mating per queen was set at 7 and 15. Non-parametric tests (Friedman's or Wilcoxon signed rank tests) were used to test for significant differences in total variance between these treatments

Table 5.1. Allele frequencies observed at microsatellite loci through two rounds of selective breeding

Locus	Alleles	G0*	G1	G2	Locus	Alleles	G0*	G1	G2
A7					**A441**				
	123	1.82	0.91			141	0.91		
	125	7.27	10.91	13.46		147	5.45	6.25	
	129	45.45	35.45	36.54		149	10.91	11.46	12.04
	131	0.91	5.45	4.81		151	29.09	14.58	23.15
	135	19.09	21.82	19.23		153	50.91	64.58	61.11
	137	4.55	11.82	5.77		155	2.73	3.13	3.70
	139	12.73	4.55	12.50					
	155	0.91							
	183	7.27	7.27	5.77					
Ap43					**A79**				
	154	15.45	18.52	22.73		110	34.55	27.27	22.73
	156	30.91	24.07	24.55		115	3.64	0.91	0.91
	158	0.93				117	4.55	0.91	2.73
	160	0.91	5.56	0.91		119	11.82	27.27	6.36
	162	11.82	16.67	10.00		121	20.91	22.73	35.45
	164	26.36	15.74	20.91		123	10.91	10.91	10.91
	166	1.82	5.56	6.36		125	5.45	3.64	11.82
	168	3.64	3.70	3.64		127	4.55	0.91	1.82
	192	0.91				129	0.91	2.73	4.55
	195	7.27	9.26	10.91		131	0.91		
	208	0.91				133	0.91	2.73	2.73
						135	0.91		

* Source population (G0) allele frequencies used for Monte Carlo simulation input.

5.2.2 csd *methodology*

The *csd* hyper-variable region (HVR) was genotyped (chapter 4) in all the samples (N= 55 per generation) collected for genetic monitoring. Results revealed 44 separate fragment lengths, far more than the minimum number of sex alleles required to maintain a bee population (Carvalho, 2001), and beyond the range of 10-20 being commonly accepted and published as expected norms (Cook and Crozier, 1995). It is very unlikely that each fragment represents a functionally unique "allele".

Therefore, to run my model, and working under the hypothesis that number of repeat units in the *csd* HVR might have an influence on function, I pooled fragment sizes into 11 classes ('alleles') (Table. 5.2), and calculated the observed frequency of each class for each generation. Division of fragment sizes into classes was arbitrary, and the resulting number of alleles and corresponding frequencies may not truly reflect actual sex allele frequencies in the population. Nevertheless, the model should provide an idea of how a population with this specific sex allele frequency configuration might respond to different selection and breeding protocols.

As in the selectively neutral model, I first ran the five generation model using a selection criteria similar to the one currently adopted by the WWBBP. I then assessed the influence of breeder numbers on the contemporary evolution of *csd* in the population. The number of randomly selected breeders was raised to eight, twelve, and twenty breeders per year, although it is unlikely that operations of comparable size to WWBBP have the resources to support more than twelve breeders each year. There is also a limit to the number of new colonies that a small breeding operation can sustain each year. The WWBBP currently uses four breeders per year, and using 20 daughter queens from each to start 60 or so (assuming ~75% mating success) new colonies. This number may be required to replace annual loss, since beekeepers have experienced above average losses over recent years. In 2012, a colony loss survey in the USA reported the fifth consecutive year of losses close to, or above 30% (vanEngelsdorp et al., 2012). Similar losses have been experienced locally (e.g., the WWBBP lost 42% of it bees over the 2010/11 winter). The program in Wales may on average need 60 new colonies per year to maintain bee numbers.

As for the selectively neutral model, a frequency variance was calculated for each simulated *csd* allele frequency distribution. For each selection scenario, the individual

allele frequency variances were totalled to give 'total sample variance' at the csd for each selection (breeding model) scenario. Total variance was calculated and compared when 4, 8, 12 and 20 breeders were used, and when 100 colonies were raised each year.

5.3 Simulation Results

5.3.1 Microsatellites

The model parameters were initially set to reflect current WWBBP protocols. The frequency shifts of 38 alleles from four selectively neutral loci (A7, A441, Ap43 and A79) were simulated and compared to observed allele frequencies after two breeding cycles. Observed frequencies were within one standard deviation of simulated means in twenty nine of thirty eight cases (Table 5.3). The simulated median allele frequency value dropped to below 0 when initial alleles frequency was low (~<0.1). By this measure, eighteen of the original 38 alleles were potentially lost due to chance as allele frequency variance increased across selection cycles (Fig 5.1).

Table 5.2 Indicating how eleven allele classes were arbitrarily assigned

	G0			G1			G2		
Allele	**Size**	**Count**	**Total count**	**Size**	**Count**	**Total count**	**Size**	**Count**	**Total count**
1	407	1	4	407	1	4	407	2	2
	415	3		415	3				
2	420	1	8	419	2	9	422	7	15
	422	2		422	5		423	8	
	423	5		423	1				
				425	1				
3	428	1	13			13	428	2	13
	429	6		429	9		429	7	
				431	2		431	1	
							433	2	
	435	6		435	2		435	1	
4	437	5	8	437	2	10	437	3	6
				438	4				
	439	3		439	4		439	3	
5			12			15	443	2	28
	446	3							
	447	1					447	8	
							448	1	
				449	2		449	4	
	450	1		450	4		450	4	
	452	3		452	8				
	453	2		453	1		453	3	
	454	2					454	3	
							456	3	
6	457	3	7	457	2	5			2
	458	4		458	3		458	2	
7	461	22	30	461	11	21	461	15	23
	463	1							
	464	7		464	10		464	8	
8	466	8	13	466	4	23	466	5	17
	467	3		467	13		467	7	
	469	1		468	2				
				469	1		469	1	
	470	1		470	3		470	4	
9	472	4	7	472	5	6			0
	475	1		476	1				
	476	1							
	478	1							
10	480	1				0			0
	485	1	3						
	486	1							
11	493	1	1	493	2	2			0
		106	**106**		**108**	**108**		**106**	**106**

Table 5.3. Observed vs Simulated allele frequency means at 4 Msat **loci**

Locus	Allele	Observed	Simulated Mean	SD
A79	***1***	***0.227***	***0.343***	***0.114****
	2	0.009	0.037	0.045
	3	0.027	0.047	0.051
	4	0.064	0.116	0.073
	5	***0.355***	***0.209***	***0.098****
	6	0.109	0.112	0.076
	7	0.118	0.055	0.054
	8	0.018	0.045	0.048
	9	0.046	0.009	0.022
	10	0.000	0.009	0.022
	11	0.027	0.010	0.024
	12	0.000	0.009	0.021
A441	***1***	***0.000***	***0.009***	***0.006****
	2	***0.000***	***0.055***	***0.014****
	3	0.120	0.109	0.020
	4	***0.232***	***0.289***	***0.028****
	5	***0.611***	***0.510***	***0.031****
	6	0.037	0.028	0.010
A7	1	0.000	0.020	0.034
	2	0.135	0.075	0.062
	3	0.365	0.448	0.118
	4	***0.048***	***0.010***	***0.022****
	5	0.192	0.189	0.095
	6	0.058	0.047	0.050
	7	0.125	0.128	0.080
	8	0.000	0.010	0.023
	9	0.058	0.074	0.061
Ap43	1	0.227	0.160	0.089
	2	0.246	0.305	0.107
	3	0.009	0.010	0.024
	4	0.100	0.117	0.077
	5	0.209	0.262	0.104
	6	***0.066***	***0.018***	***0.032****
	7	0.039	0.036	0.043
	8	0.000	0.010	0.024
	9	0.109	0.071	0.059
	10	0.000	0.010	0.024

***** The simulated frequency was more than 1 standard deviation (based on 1500 iterations) from the frequency observed in the real population

No significant difference was detected in total allele frequency variance when the size of the simulated population was reduced from 1000 to 100 individuals when 30 breeders were used per breeding cycle (Table 5.4 and Fig 5.2). Contrastingly, there was a significant difference in total variance if the population level was similarly reduced (from 1000 to 100) and only 4 or so breeders were used (Table 5.4 and Fig 5.2). There was a significant difference in the variance of the allele frequencies generated by the use of either 4 or 30 breeders per cycle regardless of the simulated population's size (100 or 1000 colonies each year; Table 5.4). Finally, the mean mating success of the simulated population queens had no statistically significant effect on total allele frequency variance ($Z = -1.153$, $P = 0.249$; Fig 5.2) when all other variables were held constant.

Fig 5.1a- Microsatellite A7

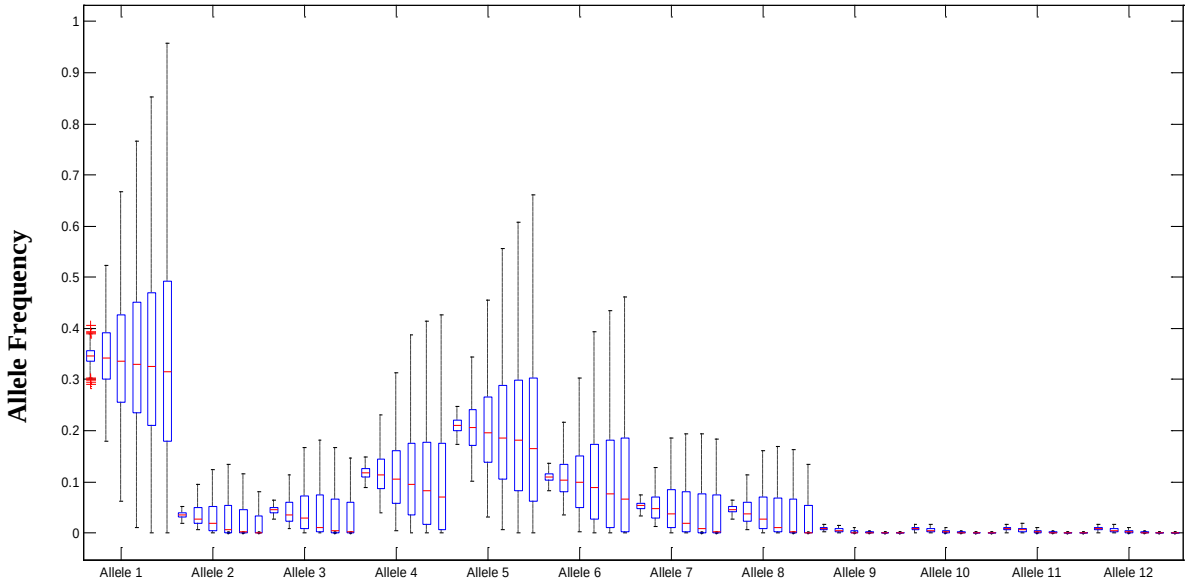


Fig 5.1.b-Microsatellite A441

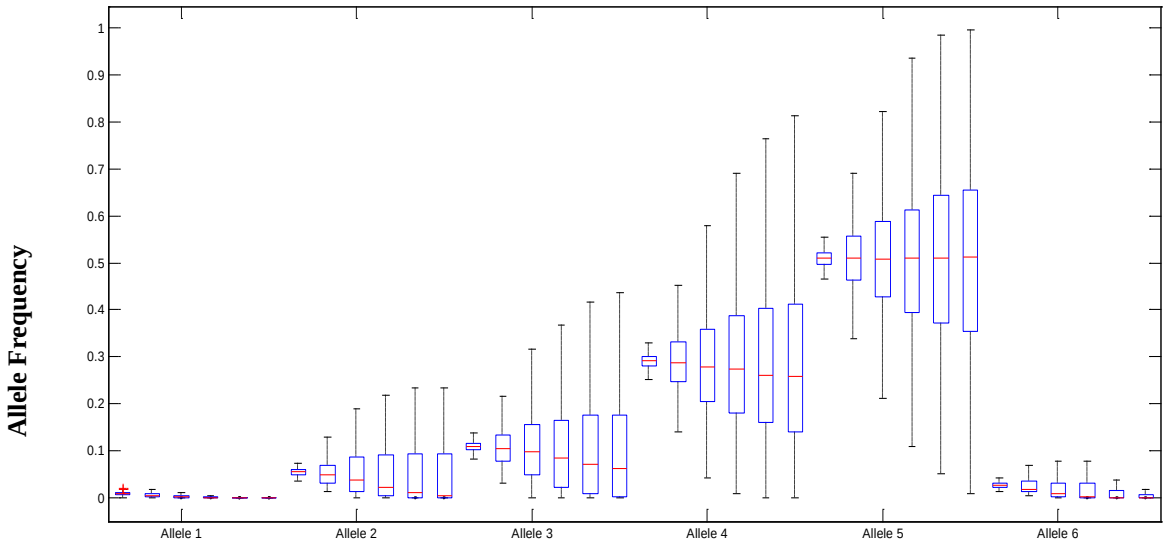


Fig 5.1c Microsatellite A79

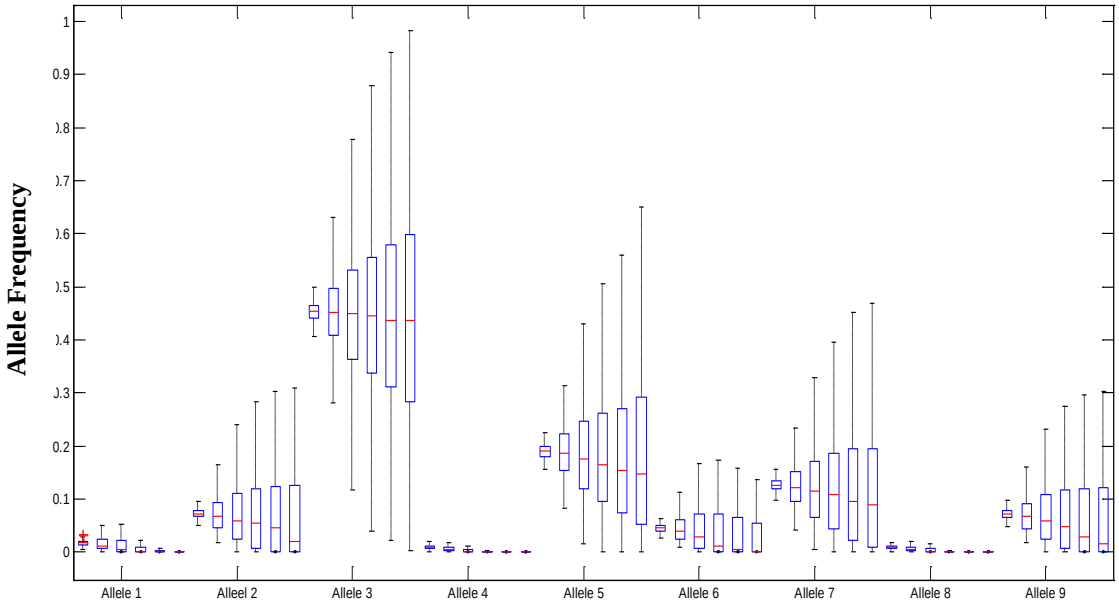


Fig 5.1d- Microsatellite Ap 43

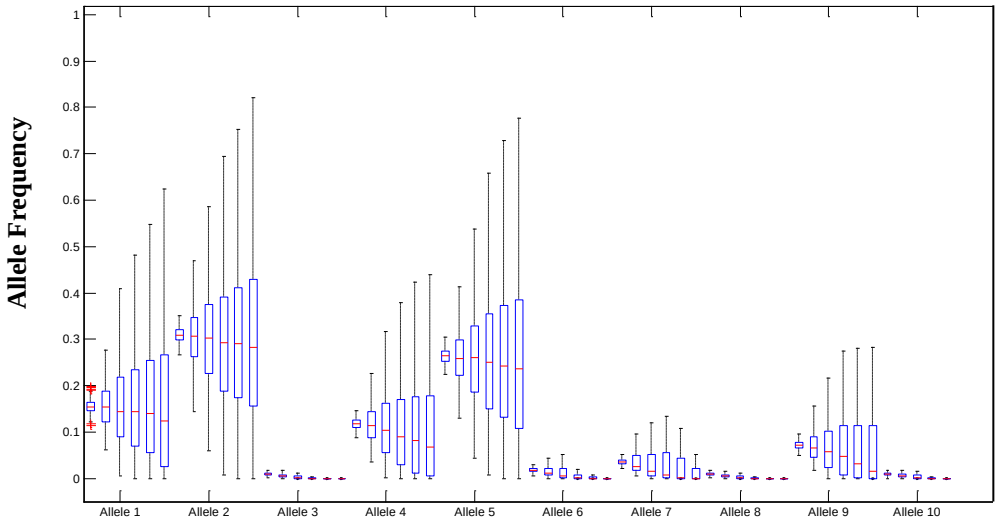


Figure 5.1. These figures (a-d) present box-plot representation of simulated changes in allele frequencies through five rounds of selection at four microsatellite loci. Model parameters were set to reflect the selection parameters currently employed by the WWBBP. The red bar across box indicates median value. The top and bottom margins of the box mark the 75 and 25 percentile, so that 50% of the results (1000-1500 iterations) fell within the box. If outliers are ignored, the remaining 50% of the results fall outside the box, but within the upper and lower limits of the overall range, as indicated by the dotted line.

5.3.2 Simulating csd (*under WWBBP protocols)*

Observed allele frequency means for ten of the 11 source population allele classes were within one standard deviation of simulated means (Fig 5.3a), and eight of the 11 observed allele frequencies means were within one standard deviation of G1 and G2 simulated means (Fig.'s 5.3b and 5.3c). Total sample variance increased with each round of selection (Fig. 5.4) and comparisons using Wilcoxon sign-rank tests indicated statistically significant differences in allele frequency variance between all paired generations (breeding cycle) ($Z = -2.934$, $p = 0.003$ in all cases). The probability of homozygosity increased to a maximum of 0.184 after five generations of selection (Fig 5.5) suggesting that a mean brood viability of 82% could be expected after 5 years. The effective number of sex alleles decreased from 7.68 to 5.44 over the same 5 cycle period. The simulation also predicts that several low frequency alleles ($\sim < 0.05$) will be lost from this population (Fig 5.6). The median allele frequency value (bar across box) dropped below or close to 0 for three low frequency alleles (Alleles 1, 10, and 11). These alleles were lost due to chance more often than not during simulation runs.

Table 5.4. Table indicating statistical differences in total allele frequency variance at neutral loci between different simulation treatments

No Daughters [Å]	No Breeders[€]	Drones	Z	P-value	Fig 5.3
1000 vs 100 [Å]	30	7	-1.153	0.249	*c and d*
1000 vs 100 [A]	4	7	-2.210	0.028*	*a and b*
1000	(8)4 vs 30[€]	7	-1.992	0.046*	*a and d*
100	(8)4 vs 30[€]	7	-2.210	0.028*	*b and c*
100	(8) 4	7 vs 15[˚]	-1.153	0.249	*b and e*

[Å] Tests for statistical difference in total allele frequency variance with daughter population sizes of either 1000 or 100 colonies were conducted when 30 or 4 breeders were used each generation.
[€] The statistical influences of different breeder numbers ((8) 4 and 30) on total frequency variance was tested in populations of size 100 and 1000 colonies. (8)4 indicate that eight breeders were initially selected from a source population, and four for each subsequent generation (as implemented by the WWBBP).
*Indicates a statistically significant difference.
[˚]The potential influence of mean mating success on the total frequency variance under WWBBP protocols was considered.

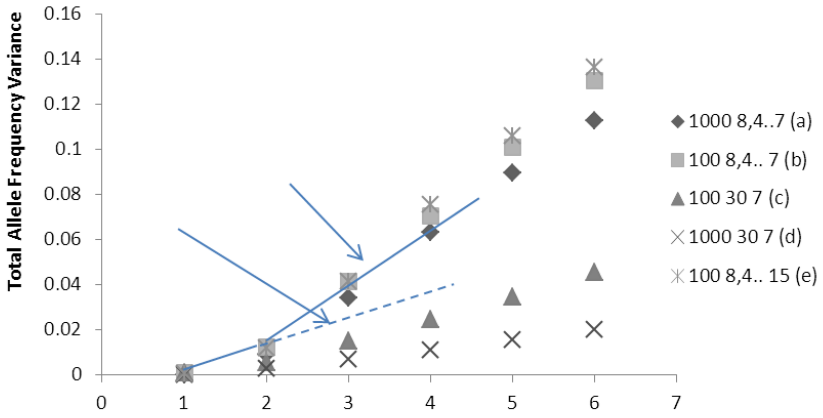


Figure 5.2 Shows change in variance at neutral autosomal loci with each selection cycle for different selection protocol (a through e). Legend indicates number of new daughter queens raised (1000 or 100), number of breeders selected each year (8 first years followed by 4 in each subsequent year; 8,4, or 30 each year) and number of drones each queen mates with (7 or 15). There was an increase in rate of change of variance when number of breeders changed from 8 to 4 (compare slopes of solid and dotted lines). **Table 5.4** indicates statistically significant differences between treatments

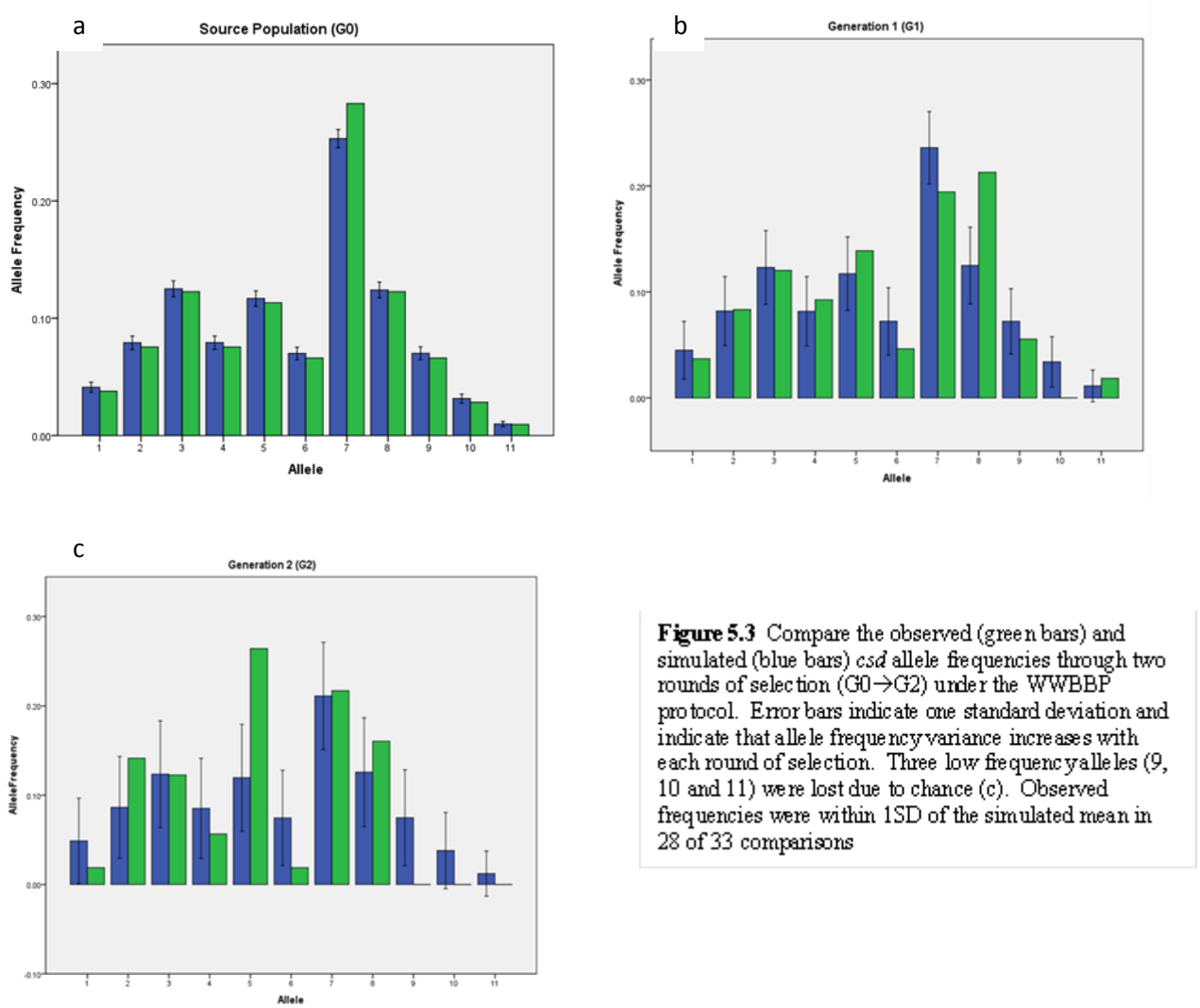


Figure 5.3 Compare the observed (green bars) and simulated (blue bars) *csd* allele frequencies through two rounds of selection (G0→G2) under the WWBBP protocol. Error bars indicate one standard deviation and indicate that allele frequency variance increases with each round of selection. Three low frequency alleles (9, 10 and 11) were lost due to chance (c). Observed frequencies were within 1SD of the simulated mean in 28 of 33 comparisons

5.3.3 Additional modelling of csd

The variance in the allele frequency data generated by Monte Carlo sampling decreased as the number of breeders selected each year increased (Fig 5.4). There was a statistically significant difference between the variance observed after five simulated generations using WWBBP selection criteria, and when either 12 or 20 breeders per year were used (Wilcoxon sign rank test; Z= - 2.197, P = 0.028 in both cases). The likelihood that sex alleles were lost due to chance correspondingly decreased as more breeders per year were used (Figures 5.4 and 5.6).

The predicted probability of homozygosity after five rounds of selection was significantly influenced by the number of breeders used, and ranged from a high of 0.183, to a low of 0.129 when either four or twenty breeders per year were used respectively (Fig 5.5). This translates into a mean brood viability of 82% after 5 rounds of selection when only four breeders are used. Regardless of mean mating success, the effective number of alleles in the population dropped from 7.4 to 5.6 over 5 years when only 4 breeders are used, and dropped to 6.6 and 7.1 over the same time period when 8 and 12 breeders were used respectively. No drop in the effective number of sex alleles was observed when 20 breeders per year were used (Table 5.5).

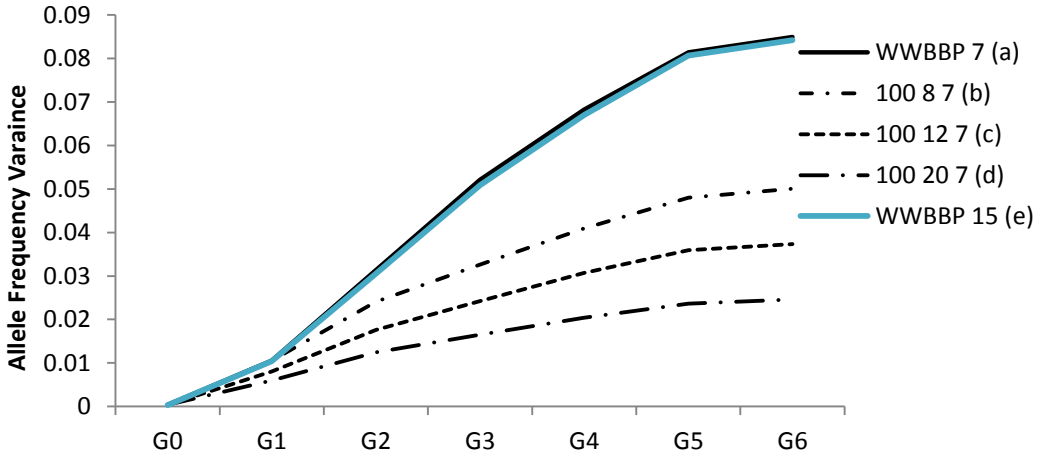


Figure 5.4 indicates the influence of breeder number on the allele frequency variance through six generations of selection. Simulations **a** and **e** indicate the protocol used by West Wales Bee Breeding Program (i.e., 8 breeders and 100 new queens from G0, followed by 4 breeders and 60 new queens for subsequent generations). Each new queen hypothetically mates with 7 or 15 drones. Simulations **b**, **c** and **d** produce 100 new queens a year and select 8, 12 and 20 breeders each year respectively. Each queen is assumed to mate with 7 drones. The figure demonstrates how increasing breeder number results in reduced allele frequency variance, and a genetically more stable population

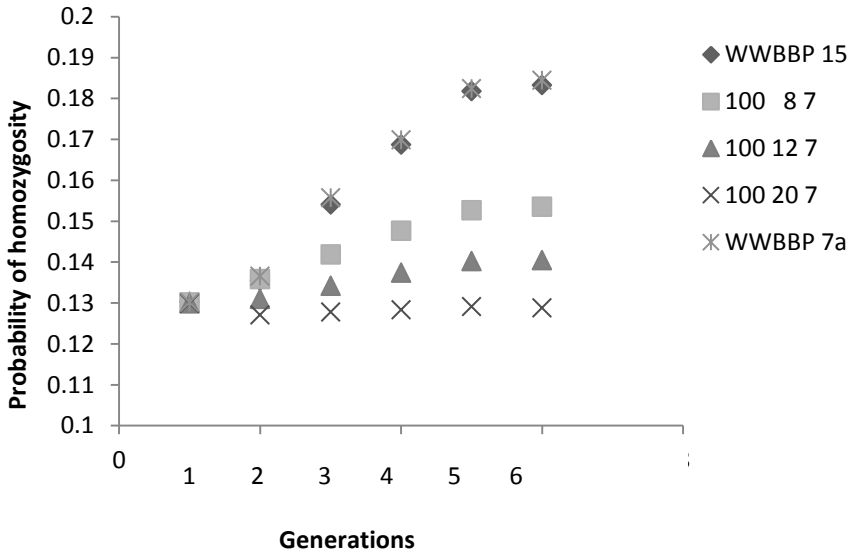


Figure 5.5 indicates how the probability of homozygosity at the *csd* increases as number of breeders (20, 12, 8, WWBBP) selected each year decreases. Increased homozygosity results in decreased brood viability (V) since V = 1 – (probability of homozygosity).

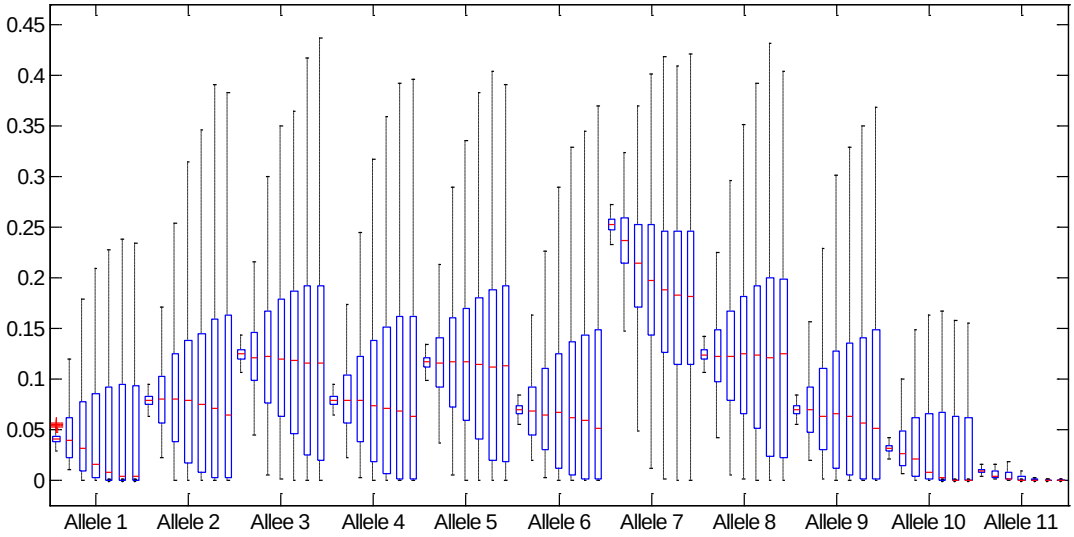


Figure 5.6. A visual representation of a *csd* dataset created using a simulation model (2500 iterations). Allele frequency variance is tracked through six successive rounds of selection (separate box-plot for each round of selection per allele) and the model parameters were set to reflect the breeding protocols currently being proposed by the WWBBP. Eight effective breeders were initially selected from a source population, and 100 or so new colonies formed. Four breeders and only 60 new colonies were formed for the remaining rounds of selection. Each new colony queen was mated with seven drones. The simulation predicts that three low frequency alleles (<0.05) will be lost from this population since the median allele frequency value (bar across box) dropped below or close to 0 (Alleles 1, 10, and 11). These alleles were lost due to chance more often than not during simulation runs. It is also noticeable that the allele 7 rapidly drops in frequency for the first four rounds of selection. Balancing selection at *csd* purges high frequency alleles as they occur more often than low frequency alleles in a homozygote state.

Table 5.5 Change in effective number of *csd* alleles through successive rounds of selection in relation to number of breeders used

	No of Breeders			
	4	**8**	**12**	**20**
G0	7.69	7.68	7.70	7.70
G1	7.32	7.36	7.63	7.87
G2	6.42	7.05	7.45	7.82
G3	5.88	6.77	7.28	7.79
G4	5.48	6.55	7.13	7.75
G5	5.42	6.51	7.12	7.76

5.4 Discussion

As expected, the number of breeders used each year had the most significant influence over the rate of loss of genetic diversity. Modelling also supported observed data showing that low frequency alleles are being lost form the WWBBP population. A significant increase in total allele frequency variance was observed when the WWBBP protocol was modelled in simulation trials, suggesting that this population might lose genetic diversity in the long term when only four breeders per year are used. These results concur with observed data acquired through genetic monitoring. In addition, modelling of population level dynamics at the *csd* (although only hypothetical in nature since actual *csd* allele frequencies are unknown) suggested that brood viability could potentially drop to below 85% within five years if current breeding protocols are maintained.

Earlier simulation work on closed population breeding considered various selection scenarios (e.g. within family, mass, and random), but in each case, only selected breeding colonies (queens) contributed towards the next generation. They were theoretical in nature, advocated the use of II, and focused mainly on simulating loss of sex alleles through time (up to 40 generations). In this study, I model small scale breeding utilizing open mating, and compare simulated results with real observed data at neutral autosomal markers. The same breeding/selection model is then applied to the sex locus model, though the input data used in this case was more theoretical in nature. These models simulated a small operation utilizing open mating with a contemporary timescale.

5.4.1 Selectively neutral markers

There was relatively strong congruence between the simulated and observed allele frequency means at three of the four selectively neutral loci modelled, with both approaches indicating that low frequency (neutral) alleles have been lost due to chance under the current WWBBP selection regime. The breeding program used approximately eight effective breeders during the first year of selection, and reduced this to four the second season. Data generated through simulation reveal a marked increase in allele frequency variance when breeder number was reduced to four. Increasing variance with each breeding cycle is also illustrated by box-plot presentations of data generated through Monte Carlo simulation. These figures also show median allele frequencies consistently dropping with each breeding round when only four breeders per cycle were used. The simulated populations lost genetic variation due to selection, and suggest that real populations might suffer a similar fate under like circumstances.

These simulations demonstrated that number of breeders per breeding cycle is the parameter that has the greatest influence on allele frequency variance at selectively neutral loci. Allele frequency variance is significantly reduced (stability increased) when number of breeders is increased. The genetic significance of differing numbers of colonies in the population is diminished in comparison. For example, no statistical difference in total variance was observed when either 100 or 1000 colonies were raised from 30 breeders each year. A difference in total variance was observed between these population sizes when only four breeder queens were used each year. This significant difference was possibly due to increased influence of male mediated input resulting from the increased number of queens being mated. Drones may have a more significant influence on population level genetic variation when queen breeder numbers are low and number of daughter queens is large. Using a small number of breeders resulted in increased variance (due to chance loss of low frequency alleles) and significantly greater genetic uncertainty, regardless of the number of colonies raised. This leads to a reduction in effective population size, possibly an inevitable consequence for small closed breeding populations

Moritz (1984), used previously published data to regress inbreeding depression on inbreeding coefficient, and then related this mathematically to population size. Using this approach, he determined that inbreeding effects can essentially be ignored until the inbreeding coefficient (F) reaches a critical value (he estimated this to be F = 0.25). How

rapidly a population reaches this value depends on its size, and is reached earlier in smaller populations. Moritz estimated that this critical value will be reached in 10 years when eight new queens each year are selected. However, his model assumed II with each selected breeder only contributing drones and queens to the next generation. This scenario differs from the open mating protocols being adopted by the WWBBP. Here, surviving daughters from the previous generation's breeder queens contribute most of the drones, and the current set of selected mother colonies (the breeders) makes no drone contribution. Nevertheless, both the observed and simulated results indicate that the genetic diversity is being lost.

Mating success had little effect on population level total allele frequency variance, and should therefore not significantly affect the genetic makeup of each generation. These simulations model open mating scenarios in which all drones from the previous year's daughter colonies have an equal chance at mating. This aspect of the model reflects the current approach being adopted by WWBBP, but may not truly reflect the situation in many breeding operations. Large scale worldwide breeding operations can raise thousands of queens per week from twenty or so breeders. Most raise daughter queens from selected colonies for use as drone contributors the following year. This way, the adaptive transfer of specific desired colony characteristics is mediated through the male as well as the female line. Isolated mating areas (with apiaries holding mating nuclei with virgin daughters of selected breeder queens) can then be flooded with these 'selected' drones so that breeding is directed from both male and female lines. Such an approach increases adaptive influence, but might be logistically demanding from smaller scale operations. Aiding adaptive change through drone influence will be less stringently controlled in such cases (as with the WWBBP). Allowing virgins uncontrolled access to drones from any number of successfully overwintered colonies might be a more practical for small scale breeders. From a breeding perspective, this approach offers limited control and lacks scientific rigor, but is advocated as a more holistic approach by some commercial beekeepers since it allows the bees be naturally selected for local adaptation. The assumption here is that drones from locally adapted queen lines with have a fitness advantage over drones from less vigorous disease prone lines, so that locally beneficial traits will be enhanced in the population.

5.4.2 CSD modelling

The *csd* simulation evolved as a progression from the selectively neutral model and was designed to simulate population level shifts in brood viability resulting from diploid male production (*csd* model). This model is complicated by the fact that no diploids can be homozygous at that locus so that all simulated diploid individuals must to be continually purged.

It is unclear how my model relates to the actual WWBBP experimental population with regards to the frequency dynamics of functionally specific *csd* alleles. Great variation in fragment length was detected in the HVR, a region thought to possibly confer functionality at *csd*. It is unclear how one functional allele differs from another, but based on these data, it seems unlikely that length is the determining factor. There are estimated to be 19 or so distinct *csd* alleles (Adams, 1977), but twice as many HVR fragment lengths were found in this small population alone. Replication errors might possible lead to high mutation rates at the repetitive HVR sequences, and variants are maintained over time by balancing selection acting on functional determining characteristics (e.g. non-synonymous single nucleotide mutations).

The HVR range in fragment sizes were arbitrarily assigned into the eleven designated 'allele classes'. Consequently, the frequency distribution entered into the model is purely hypothetical in nature. Nevertheless, there was relative congruence between simulated and observed means through two rounds of selection. Both approaches indicated that *csd* 'alleles' at frequencies below 0.05 are vulnerable to loss due to chance under current WWBBP selection protocols. Observed data (genotyping) indicated that three low frequency fragment size categories (arbitrary alleles) were lost due to chance after two rounds of selection.

Simulating progression through an additional three breeding cycles using current WWBBP protocols shows that the median values for alleles found at frequencies less than 0.1 in the source population, continued to fall with each successive round of selective breeding. This result suggests that a population with this specific *csd* allele frequency distribution would be genetically unstable at this locus for a few years under these particular selection parameters. Probability of homozygosity at *csd* increases, and the effective number of alleles in the population decreases as genetic diversity is lost to chance. However, there is evidence that an equilibrium state might be achieved within a few cycles. The rate of change in allele frequency variance decreases after five breeding

cycles, and there is a corresponding levelling off in probability of homzygosity and number of effective alleles in the population

Page and Laidlaw (1985) present simulation data indicating a similar trend. Starting with a large population, and raising large numbers of colonies each year, they graphed the probability that brood viability would be greater than 85% against number of generations for differing numbers of selected queens each year. Ten was the smallest number of breeders they simulated. In this case, the probability that brood viability would be over 85% rapidly dropped from near 1.0 to 0.2 in five generations. Thereafter the rate of change declined with each generation. Similar trends are observed for each scenario they simulated, and in general, selecting more breeders reduced the inevitable decline in brood viability. They also report that at least 50 breeders must be selected from each generation to maintain a 95% probability of at least 85% brood viability after 20 generations.

A progression towards an apparent equilibrium state within five generations is similarly observed in my simulations. As equilibrium is approached, allele frequency variance and probability of homozygosity decrease as the numbers of breeders used increases. This trend towards equilibrium is highlighted by the observable shifts in the median allele frequency values across generations. High frequency allele medians (e.g. allele 7) drop as homozygotes carrying these alleles are purged from successive generations. Lower frequency alleles are then favoured and tend to increase in frequency. The model results reflect balancing selection on the *csd* locus, and highlight the influence of breeder numbers on the amount of variation that a population can maintain. Populations can maintain higher effective numbers of alleles when more breeders are used. This makes sense as effective population size is defined by number of breeders contributing to next generation. More breeders result in larger effective population sizes which can maintain more genetic variation.

5.4.3 Summary/Recommendations

Small bee breeding operations have more control over number of breeders used than any other breeding parameter. Number of new colonies established each year is limited by resource availability, and the mean mating success of daughter queens is largely weather dependent. The WWBBP can generally start no more the 100 new colonies a year in Wales; they used 4 breeder queens last year, and tried to raise 20 daughter queens from each one. In addition, mating success may have been sub-optimal over recent years due to

persistent and prolonged periods of poor weather. I modelled the various parameters and adjusted the number of contributing breeder queens to investigate its influence on population level diversity at both selectively neutral loci and at the *csd*. The simulations illustrate that number of breeder queens used is the parameter that has the greatest influence over the genetic makeup of future generations. Both the observed and the simulated results suggest that limiting breeder queen number to only four per year results in genetic depletion over time. Loss of diversity within a contemporary framework was observed at both selectively neutral loci, and at loci under the influence of selective forces. This suggests that loss of adaptive potential due to chance loss of adaptively significant quantitative trait loci could be expected within a contemporary time frame. There are practical implications for bee-breeders since selecting for adaptive 'progress' becomes less effective as population level genetic diversity drops. Consistently and stringently limiting genetic transfer across generations by using low numbers of breeder queens will dampen the long term vigour and health of the population. Breeders must keep this in mind when designing a breeding program.

Sex allele diversity has been a concern for breeders for many years, and particularly for those working with small populations. My model parameters were hypothetical in terms of numbers and frequencies of alleles. I modelled eleven alleles in total (an expected population number) thought the effective number was closer to 8 when accounting for differential frequencies. There were consistent drops in median allele frequencies at both the *csd* and at microsatellite loci when only four breeders were used, and <85% brood viability brood viability was attained after 5 generations. Median allele frequency dropped less with eight, and less again with twelve breeders. Median values stabilized when 20 breeders per year were used, suggesting that the genetic population size would be sufficiently large to maintain that many alleles over time. From a practical perspective, this whole debate boils down to one question; how many breeders should be (or can be) selected? Twenty is more than most want to manage; the results presented in this chapter suggest that a compromise of between 10-12 breeders may be feasible.

Bee breeders have ultimate control over the number of breeder queens selected each year, and modelling illustrates that of all possible variables, the number selected has the most significant influence over the long term genetic stability of a population. Simulations suggest that the WWBBP could benefit from increasing breeder numbers in order to limit the loss of low frequency alleles. Increasing the number of breeders (queen lines) would

help maintain adaptive potential, and limit the production of diploid drone production due to homozygosity at the *csd* in the long term. Evidence suggests that small scale programs should aim to maintain eight breeding queen lines in order to reduce the likelihood of rapidly losing diversity due to chance.

Chapter 6

Investigating population level *csd* variation

6.1-Introduction

All Hymenoptera (bees, wasps, ants and sawflies) lack sex chromosomes (as seen in *Drosophila*) and employ instead a haplo-diploid sex determination system. Sexual development in this case is directed by a specific region of chromosome 3 (the Sex Determination Locus - SDL), and specifically, by the complementary sex determination gene (*csd*) found within it (Beye et al., 2003). The *csd* genes translate into atypical forms of SR- proteins, an important class of gene expression regulators (Long and Caceres, 2009). SR proteins classically have a serine "S" and arginine "R" rich region (RS domain) and can mediate the splicing of precursor messenger RNA (pre-mRNA) into mRNA (after cutting out introns). They also typically have RNA binding sites, but these are lacking in *csd* (Evans et al., 2004). These SR-*type* proteins are known to interact with RNA in other ways (Long and Caceres, 2009). RS domains are frequently involved in protein-protein interaction, hence it is thought that functionally different *csd* proteins combine to form an active RNA splicing product that can activate a downstream response in another gene within the SDL (*fem*) to produce female specific mRNA (Gempe et al., 2009). Otherwise, default male specific *fem* mRNA is produced. Only individuals that are heterozygous at *csd* develop into females.

The *csd* gene comprises of nine exons distributed across a 9 kb region within the SDL. These combine to generate a 1.4kb transcript (Heimpel and de Boer, 2008) that contains a number of regions with distinct amino acid sequence characteristics (Figure 4.1). The region rich in arginine (R) and serine (S) spans exons 5–7, and a hyper-variable region (HVR) comprising mostly of asparagine (N) and tyrosine (Y) is located across exons 7 and 8. It is characterized by an $\{(N)_{1\text{-}4}Y\}n$ repeat rich region, and is bordered by a proline-rich (P) C-terminus (Gempe et al., 2009).

The basis of the difference between functional alleles is not yet understood, but the repetitive repeat region within the HVR is the prime candidate location for conferring (Beye et al., 2003; Hasselmann and Beye, 2004; Cho et al., 2006; Gempe et al., 2009) or at least adding to (Hasselmann et al., 2008) the specificity of alleles. Specificity could be due to single amino acid differences in the HVR. It is also known that repeat sequence polymorphism within amino acid coding regions can bestow allele specificity (Fondon and Garner, 2004), hence specificity might also be conferred by differing numbers of $\{(N_{1\text{-}4})Y\}_x$ repeats within the HVR. Such amino acid repeat sequences are commonly found within eukaryotic proteins (Mularoni et al., 2010) and are usually encoded by tri-repeats in

regions of high mutation rates that probably result from replication slippage (Mularoni et al., 2010), or maybe unequal cross-over during recombination. There is also the possibility that both single amino acid differences and repeat polymorphism work in combination to confer specificity. A great deal of repeat sequence variation has been observed at the putatively 'functional' HVR, and it is currently thought that different alleles differ greatly in sequence. Hasselmann and Beye (2004) sampled four *Apis mellifera* populations (one each in Germay, South Africa, United States and Brazil) and detected 15 separate *csd* lineages, each one differing by 3% in sequence variation. Around19 different forms of *csd* are thought to occur in *Apis mellifera* as a whole (Adams, 1977).

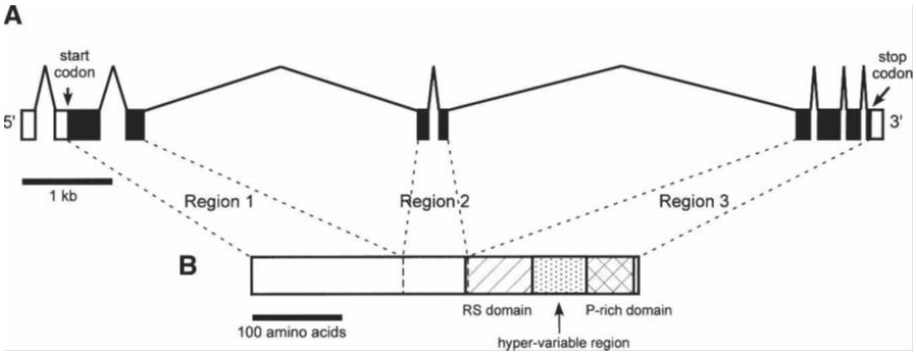


Figure 6.1 The *csd* gene has nine exons combine to produce a 1.4Kb transcript B. The hyper-variable region is found in region 3, and area that incorporates exons 6-9.
(From Cho et al., 2006)

6.1.1 Implication for breeders

Drones develop from unfertilized eggs and receive their full genetic complement exclusively from their mother. They are haploid and carry only one copy of the *csd* gene. Females in contrast develop from fertilized eggs, but will only do so successfully when the paternal and maternal *csd* alleles are different. Individuals developing from eggs fertilized by sperm carrying functionally identical *csd* alleles will develop as sexually inviable diploid (hemizygous) males. Diploid drones constitute a resource drain, and are sacrificed by colony workers. The population dynamics of *csd* can influence genetic health at the individual, colony and population level, and is therefore of imperative importance to the bee breeder. Genetically depleted populations can have reduced mean colony level brood viability (less productive) due to increased diploid drone production. Line breeding (in the strictest sense) is especially prone to genetic depletion as diversity is lost from closed populations due to chance. It would be of benefit to breeders to monitor *csd* variation, and to then use breeder queens of different *csd* lineages in their program. This cannot be easily done, since we still don't definitively know what differentiates one allele from another.

6.1.2 Population screening

I explored three methods to possibly assay a population for *csd* variation, and assumed in doing so that the HVR confers allelic specificity. In Chapter 4 I described a method for screening for HVR size variation, and mention the methodology and results again briefly below. In this chapter I present HVR population level haploid data. Drones carry only one copy of the *csd* gene, and hence provide easy access to allele specific sequences, and a possible way to monitor population level variation. Finally, I attempted to use denaturing gradient gel electrophoresis (DGGE) to separate HVR *csd* fragments amplified in workers. Diploid derived alleles are most commonly separated by cloning individual allele fragments into plasmid DNA. The process is time consuming and expensive. DGGE offers an alternative inexpensive approach to visualize allele specific differences amplified in diploids, and offers an alternative approach to population screening. The process uses electrophoresis to separate products based on sequence differences rather than on fragment size. The goal was to separate fragments so that they could be excised from the gel and sequenced, but I failed to get sufficient resolution to identify individual fragments (methods in Appendix v). I therefore attempted improve fragment resolution by running out the pre DGGE PCR product on low agarose melting gels, and excising the target product. Unfortunately, too much DNA was lost in the recovery process to warrant proceeding further. Although I failed to develop a working protocol, the approach does show promise, and further development work is warranted.

Genotyping previously demonstrated extreme population level fragment length diversity across the *csd* HVR. I explored the nature of this variation by sequencing the HVR in haploid males, and hypothesized that deferring numbers and combinations of $(N)_{1\text{-}4}Y$ repeats would be the most likely source of the observed diversity. I also compared my sequence results with previously reported data (Hasselmann and Beye, 2004; Cho et al., 2006; Liu et al., 2011) and used a Neighbour-Joining approach to investigate how local sequences (i.e. from the WWBBP population) clustered in relation to putative functional allele sequences derived from individuals that had been sampled from a broad geographic distribution. I expected that most putative allele sequence lineages to be represented in the local population.

6.2 Methods

6.2.1 Sequencing haploids

Drones were sampled during a single event at a local drone congregation area (situated 900 metres from the mating apiary at Glaspwll) for a related study. It is not clear how representative such a sample is of actual population diversity, but results should at least provide an indication of the minimum amount of local variation. Ninety three drones were sequenced using primer pairs previously used by (Hasselmann et al., 2010) genoRfw 5'-AGACRATATGAAAAATTACACAATGA-3', and conscsdrev 5'-TCATCTCATWTTTCATTATTCAAT-3'. These primers amplified a 750 bp (approx) fragment of coding and non-coding DNA bridging the HVR (Fig 6.1). Bi-directional sequencing was initially performed, but as extremely good sequence coverage was possible sequencing in one direction only, most samples were only sequenced in one direction.

6.2.2 Definition of csd *alleles*

Nucleotide sequence alignments were performed with ClustalX version1.8 in Mega5 (Tamura et al., 2007) and alignment results were also adjusted manually for obvious alignment errors. I also used MEGA5 to compare the drone derived *csd* sequences using a Neighbour-Joining (NJ) approach (Saitou and Nei, 1987). The NJ method seemed appropriate since no phylogenetic inference was intended. In a similar way to Hasselman and Beye (2004), and Liu et al., (2011) (who investigated possible founder effects by examining *csd* region3 variation in an island population of a related species, *A. dorsata*), a representative sample from each resulting sequence lineage (cluster) was considered as a distinct allele and compared to the coding region sequences of previously published putative alleles (Hasselmann and Beye, 2004). These authors sampled 200–300 embryos from two to three *A. mellifera* colonies from four geographical locations: Davis (CA), Berlin, Stellenbosch (South Africa), and Ribeirão Preto (Brazil). They had a geographically diverse sample set, and although only a few colonies were sampled, the samples were expected to be genetically diverse due to polyandry. That is, these samples carried the genetic contribution of many different fathers (Palmer and Oldroyd, 2001). The coding regions were determined by consulting the *A. mellifera csd* gene sequence reported by Hasselmann and Beye (2004) and Cho et al. (2006), and cDNA sequences of the *A. mellifera csd* gene reported by Beye et al. (2003). The coding frame (no stop codons) was also confirmed using the alignment program CodonCode Aligner. I used Arlequin (v3.5.1.3; Excoffier and Lischer, 2010) to assess haplotype variability and to assess degree of gene diversity in the population. This measure is defined as the average

number of nucleotide differences per site between any two DNA sequences chosen randomly from the sample population, and is denoted by π.

I also used NJ to compare haploid derived fragments of known length to see if they clustered into separate sequence-based lineages. If so, then screening for population level HVR fragment size variation as determined by genotyping (can use easily sampled diploids for this) could indicate how much *csd* variation the population is carrying.

6.2.3 *Genotyping*

I used genotyping methodology (as described in Chapter 4) to investigated *csd*-HVR fragment length polymorphism. A primer pair was developed to target conserved regions on either side of the HVR, and designed so that fragment lengths would be approximately 450 base pairs (bp) long to facilitate standardizing with the ABI Genescan500 size standard. The methodology could be applied to both diploid and haploid individuals (see below), and was used to track genetic change due selective breeding in managed a bee population.

6.2.4 Sequencing diploids

Worker samples were taken from each of three generations for monitoring purposes, and a number of individuals were found to carry HVR fragments of equal lengths. These individuals became the focus of additional attention since a difference in the number of $(N_{1\text{-}4})$Y repeats at the HVR (resulting in difference in size) is a proposed mechanism of conferring *csd* allele specificity. The detection of *csd* heterozygous individuals with HVR fragments of equal lengths could be explained in one of two ways; either the two HVR 'allele' sequences were in some way different, or alternatively, if the separate allele sequences are identical, the HVR performs no functionally specific task during sex determination. Simply sequencing genomic DNA from 'homozygous' (in terms of fragment size) individuals might shed light on this question

In order to expand sequence coverage either side of the HVR, I then amplified the HVR of diploids previously determined to have identical fragment lengths using my genotyping primers (approx.450 bp coverage), with the primer pair used by Hasselman et al., (2010). These primers provided approximately 750bp of coverage across the region. Constructing allele specific sequences from heterozygotes using genomic DNA can be problematic since both alleles will be amplified, and resolving which base goes with which variant

problematic. This was not an issue here as I was only interested at this stage in whether there were sequence differences at the HVR between allele variants, and not in reconstructing specific allele sequences. I used CodonCode Aligner to align sequences and preserve coding frame. MEGA5 was also used for aligning noncoding sequence and for analysing the data.

6.3 Results

Thirty-four different sequence haplotypes were identified from 95 drones sampled at local congregation areas. Thirty-three distinct haplotypes remained when the HVR was removed from the analysis. Approximately 750 base pairs of useful sequence were obtained using single direction sequencing. This amplified fragment comprised of coding and non-coding regions spanning either side of the *csd* HVR. The gene diversity per locus (π), across this whole sequence, was 0.05 ± 0.02, and Neighbour–Joining (NJ) clustering of sequence data revealed around 20 distinct lineages (Fig 6.2). Very similar clustering trends were observed when the HVR (region with multiple gaps) was removed from the analysis. Representative samples from each lineage were aligned at the csd-HVR region (Fig 6. 3). The HVR coding regions ranged in size from 69 to 90 amino acid residues and demonstrated comparable variation to those published by Hasselman and Beye (2004) (Fig 6.4).

Genotyping revealed twelve diploid workers carrying HVR 'alleles' of equal lengths. Sequences were found to be different between alleles in all cases and markedly so in eleven of the twelve cases (sequence electropherograms were unintelligible in these cases). Contrastingly, one individual (esg3312) carried two very similar coding region sequences at the HVR. Only six single base pair differences were observed across the 454 bases spanning the HVR coding region. Three of these differences were synonymous, and two of the remaining three non-synonymous trasversions were located within the HVR (Figure 6.4). One of these single nucleotide substitutions (G<->T) was located in RS domain and resulted in a Threonine (T) to Arginine (R) amino acid mutation at position 302 (Cho et al., 2006). Of all the haploid individuals sequenced (~100), this R variant was uniquely observed. The other two mutation were A<-> T trasversions, found within the HVR. The resulting amino acid sequences had either an Asparagine (N) or Lysine (K) at position 340, and an N or Tyrosine (Y) at position 347 (Cho et al., 2006).

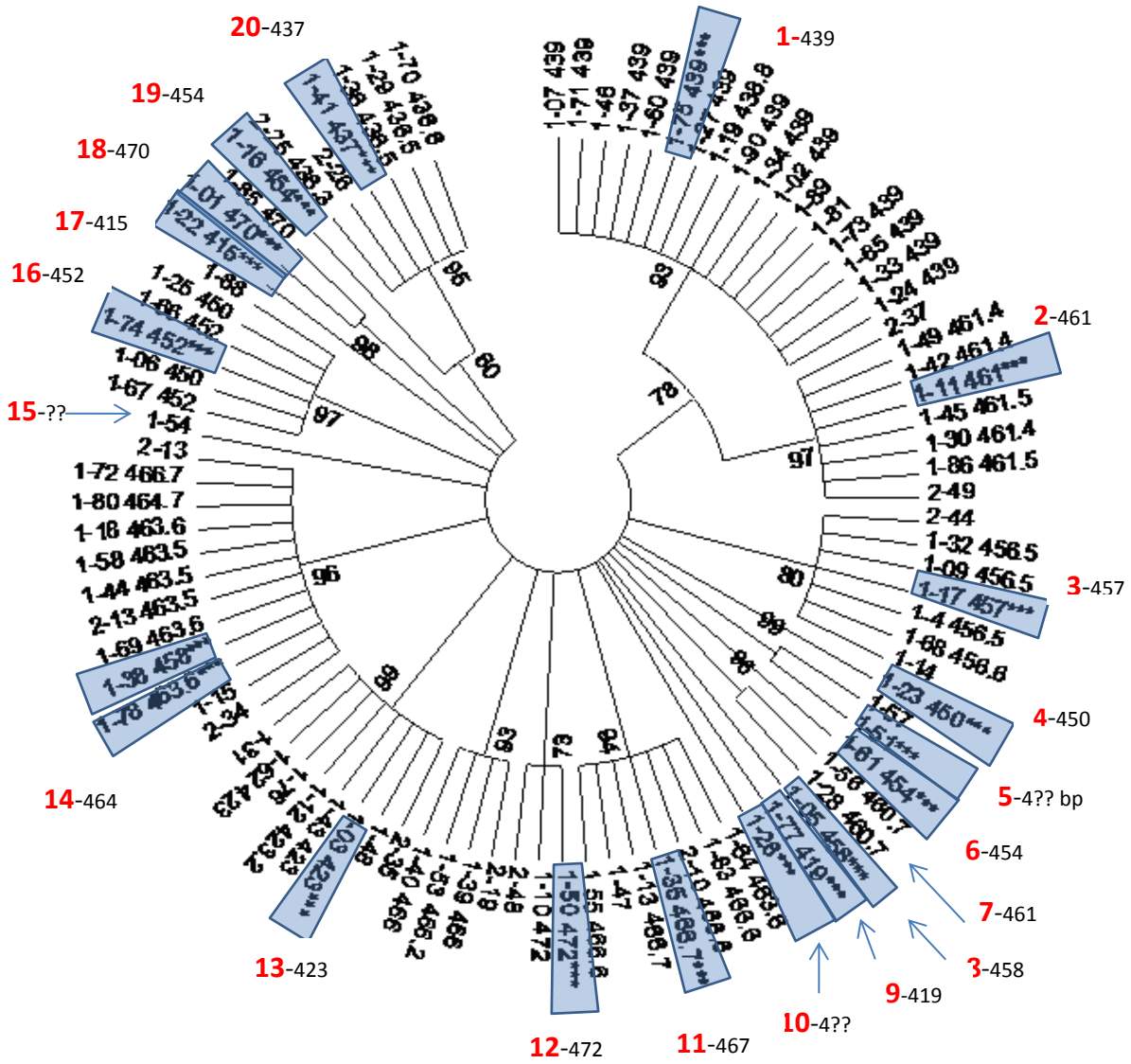


Figure 6.2. Around twenty distinct lineages (**1-20**) were identified by Neighbour-Joining clustering. Lineage designation generally correlated with genotyped fragment size, though this relationship was not exclusive. For example, lineage **8** is comprised of a single individual with a genotyped fragment size of 458 bp's. An individual (sample 1-38) of the same size clustered into lineage **14**. Similarly, individual 1-72 also clustered into lineage **17** but is of similar size to lineage **11** (i.e.467). The apparent relationship between fragment size and lineage suggests that an indication of population level *csd* variation might be gleened by simply screening randomly sampled drones for size variation across the HVR. The amino acid sequences of representative individuals taken from each of the twenty lineages (blue box and ***) were compared to data previously published by Hasselman and Beye (2004; see Fig. 6.4). Remarkable variation was demonstrated in this population. The evolutionary history was inferred using the Neighbor-Joining method (Saito and Nei, 1987). The optimal tree with the sum of branch length = 0.48389041 is shown. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site (bootstrapped 2000 times). The analysis involved 92 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 187 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al, 2007).

```
#1-DCA151           HIKMRILIEN RETSKERSRD RKEQERSKEP KIISSLSNKT IHNNNNYNNN YNNYNNNYKY NYNNYKKLQY -YNINYIEQI PVPIPVPVYC G
#2-DCA178-464       HIKMKENIEN RETSKERSRD RTERERSREP KIISSLSNKT IHNNNNYKYN YNNNNNNYKN YNNY-KKLYY --NINYIEQI PVPVPV--YY G
#3-DCA150-472       HIKMRILIEN RETSKERSRD RTERERSKEP KIISSLSNKT IHNNNNYKYN YNNNYNNNHY NNNY-KKLQY -YNIINIEQI PVPVPVPIYC G
#4-DCA101-470       RIKMKKNIEN RETSKERSRD RTERERSREP KIISSLSNKT IHNNNNYKYN YNNKYNYNNN NYN--KKLYY KNYIINIEQI PVPVPV--YY G
#5-DAC135-466       HIKMRILIEN RETSRERSRD RRERERSKEP KIISSLSNKT IHNNNNYKNY NNYNNNNYKN YNY--KKLYY --NIINIEQI PVPVPVPVYC G
#6-DCA117-457       HIKMRILIEN RETSKERSRD RTERERSKEP KIISSLSNNY NYNNNNYNNY NNNYNNYNNN YN---KKLYY --NINYIEQI PVPVPI--YC G
#7-DCA204-457       RIKMKILIEN RETSKERSRD RTERERSREP KIISSLSNKT IHNNNNYNNN NYNNYNNNYN NY---KKLYY --NIINIEQI PVPVPVPIYC G
#8-DCA138-458       HIKMKENIEN RETSKERSRD RTERERSREP KIISSLSNKT IHNNNNYKYN YNNNNYKNYN NY---KKLYY --NINYIEQI PVPVPV--YY G
#9-DCA111-461       HIKMRILIEN RETSRERSRD RTERERSKER KIISSLSNNY NYSNYNNYNN NNNYNNNNYN Y----KKLYY --NINYIEQI PVPVPVPIYC G
#10-DCA105-458      HIKMRILIEN RETSKERSRD RKERERSKEP KIISSLSNNY KYSNYNNYNN YNNNNYNNYN -----KKLYY KNYIINIEQI PVPVPI--YC G
#11-DCA161-454      HIKMRILIEN RETSKERSQD RTERERSKEP KIISSLSNNT IHNNNYKYNY NNNNYNNNYN -----KKLYY KNYIINIEQI PVPVPV--YY V
#12-DCA123-450      HIKMRILIEN RETSKERSRD RTERERSREP KIISSLSNKT IHNNNNYKYN YNNNYNNNS- -----KKLYY --NINYIEQI PVPVPI--YC G
#13-DCA116-455      RIKMKENIEN RETSKELSQD RTERETSKEP KIISSLSKNT IHNNNYKYNY NNNNYNNS-- -----KKLYY --NINYIEQI PVPVPVPIYC G
#14-DCA174-452      RIKMKENIEN RETSKERSRD RMERERSKEP KIISSLSNKT IHNNNNYNNN NYNNYNN--- -----KKLYY --NINYIEQI PVPVPVPIYY G
#15-DCA141-437      RIKMKENIEN RETSKERSRD RRERKRSREP KIISSLSNHY NYNNNKYNNY NNDY------ -----KKLYY --NINYIEQI PIPVPI--YC G
#16-DCA175-439      HIKMRILIEN RETSKERSRD RTERERSREP KIISSLSNNY KYSNYNNNNY NNNS------ -----KKLYY --NINYIEQI PIPIPVPIYC G
#17-DCA126-         HIKMRILIEN RETSRERSRD RKER-RSKER KIISSLSNNY ISNISNYNNN NNS------- -----KKLYY --NINYIEQI PVPIPVPVYC G
#18-DCA177-419      HIKMKILIEN RETSKERSRD RRERERSKES KIISSLSNNY NYNNCNYKHN ---------- ------KLYY --NIINIEQI PVPVPI--YC G
#19-DCA103-423      RIKMKILIEN RKTSKERSRD RTERERSKEP KIISSLSNNY NYSNYNNNNY ---------- -----KQLCY --NINYIEQI PVPVPV--YY G
#20-DCA122-415      RIKMKENIEN RETSKERSRD RTERERSKEP KIISSLSNNT IHNNNYN--- ---------- -----KKLYY --NINYIEQI PIPVPV--YY G
#21-esg3312he_1-415 RIKMKENIEN RERSKERSRD RTERERSKEP KIISSLSNKT IHNNNNY--- ---------- -----KKLYY --NINYIEQI PIPVPV--YY G
#22-eSg3312he_2-415 RIKMKENIEN RETSKERSRD RTERERSKEP KIISSLSNNT IHNNNYN--- ---------- -----KKLYY --NINYIEQI PIPVPV--YY G
```

Figure 6.3. Aligned (samples # 1-20) are hypervariable region (HVR) amino acid sequences obtained from haploid drones randomly sampled in the test population. Each sequence is a representative of a separate lineage identified by NJ clustering (blue boxes in Fig 6.2). The figure reveals the source of much of the size variation observed by genotyping. Samples #21 and 22 are putative allele sequences from a diploid individual (esg3312). Nearly identical sequences were obtained, but electropherogram data indicated heterozygozity (double peaks) resulting in non-synonomous substitutions at three locations. These substitutions (T, N and Y variants highlighted) were arbitrarily assigned to sequence 2. The three digit numbers associated with sample identification indicate genotyped fragment size when available. Red and blue regions indicate the location of the SR domain and the Proline rich region bounding the HVR respectively.

```
#1  B1-4                  EPKIIS---S LSNKTIHNNN NYKYNYNNNN YNNNNYNNNY NNNCKKL-YY N--IINIEQ- --P
#2  DCA151-               EPKIIS---S LSNKTIHNNN NYNNNYNNYN NNYKYNYNNY ----KKLQYY N--INYIEQI PVP
#3  DCA178-463.6          EPKIIS---S LSNKTIHNNN NYKYNYNNNN NNYKNYNNY- ----KKL-YY N--INYIEQI PVP
#4  DCA101-470            EPKIIS---S LSNKTIHNNN NYKYNYNNKY NYNNNNYN-- ----KKL-YY KNYIINIEQI PVP
#5  DCA135-466.7          EPKIIS---S LSNKTIHNNN NYKNYNNYNN NNYKNYNY-- ----KKL-YY N--IINIEQI PVP
#6  DCA204-456.5          EPKIIS---S LSNKTIHNNN NYNNNNYNNY NNNYNNY--- ----KKL-YY N--IINIEQI PVP
#7  DCA138-458            EPKIIS---S LSNKTIHNNN NYKYNYNNNN YKNYNNY--- ----KKL-YY N--INYIEQI PVP
#8  DCA123-449.5          EPKIIS---S LSNKTIHNNN NYKYNYNNNY NNNS------ ----KKL-YY N--INYIEQI PVP
#9  DCA161-454            EPKIIS---S LSNNTIHNNN -YKYNYNNNN YNNNYN---- ----KKL-YY KNYIINIEQI PVP
#10 DCA116-454.6          EPKIIS---S LSKNTIHNNN -YKYNYNNNN YNNS------ ----KKL-YY N--INYIEQI PVP
#11 DCA174-452            EPKIIS---S LSNKTIHNNN NYNNNNYNNY NN-------- ----KKL-YY N--INYIEQI PVP
#12 DCA122-415            EPKIIS---S LSNNTIHNNN YN-------- ---------- ----KKL-YY N--INYIEQI PIP
#13 esg3312_seq1 415      EPKIIS---S LSNKTIHNNN NY-------- ---------- ----KKL-YY N--INYIEQI PIP
#14 esg3312_seq2 415      EPKIIS---S LSNNTIHNNN YN-------- ---------- ----KKL-YY N--INYIEQI PIP
#15 S7-58                 EPKIIS---S LSNNTIHNNN YN-------- ---------- ----KKL-YY N--IINIEQ- --P
#16 B2-25                 EPKIIS---S LLNNTIHNNN NY-------- ---------- ----KKL-QY YN-INYIEQ- --P
#17 A1-18                 EPKIIS---S LSNKTIHNNN NYNNYNN--- ---------- ----KKL-YY N--INYIEQ- --P
#18 A-58                  EPKIIS---S LSLKTIHNNN NYKNYN---- ---------- ----KKL-YY N--IINIEQ- --P
#19 DCA126-               ERKIIS---S LSNNYISNIS NYNNNNNS-- ---------- ----KKL-YY N--INYIEQI PVP
#20 DCA111-461.3          ERKIIS---S LSNNYNYSNY NNYNNNNNYN NNNYNY---- ----KKL-YY N--INYIEQI PVP
#21 DCA117-457            EPKIIS---S LSNNYNYNNN NYNNYNNNYN NYNNNYN--- ----KKL-YY N--INYIEQI PVP
#22 A2-88                 EPKIIS---S LSNNYNYNNN NYKYNYNNYN ---------- ----KKL-YY KNYIINIEQ- --P
#23 A1-28                 EPKIISNNNS LSNNYNYNNN YNNYNKHNYN ---------- -----KL-YY N--INYIEQ- --P
#24 DCA141-436.7          EPKIIS---S LSNHYNYNNN KYNNYNNDY- ---------- ----KKL-YY N--INYIEQI PIP
#25 DCA177-419            ESKIIS---S LSNNYNYNNC NYKHN----- ---------- -----KL-YY N--IINIEQI PVP
#26 DCA103-423            EPKIIS---S LSNNYNYSNY NNNNY----- ---------- ----KQL-CY N--INYIEQI PVP
#27 D2-38                 EPKIIS---S LSNNYNYNNY NNNY------ ---------- ----LPL-HY N--INYIEQ- --P
#28 S2-31                 EPKIIS---S LSNNYNYNNY NNNY------ ---------- ----KPL-YY N--IIYIEQ- --P
#29 D1-22                 EPKIIS---S LSNNYKYSNY NNYNNYNNNN YNHYN----- ----KKL-YY KNYIINIEQ- --P
#30 S2-33                 EPKIIS---S --NNYNYKNY NNNYNS---- ---------- ----KKL-YY N--IINIEQ- --P
#31 D1-18                 EPKIIS---S LSNNYKYSNY NNYNNNYNNY NNYNNNYNNN Y----KL-YY N--INYIEQ- --P
#32 DCA105-458            EPKIIS---S LSNNYKYSNY NNYNNYNNNN YNNYN----- ----KKL-YY KNYIINIEQI PVP
#33 DCA175-439            EPKIIS---S LSNNYKYSNY NNNNYNNNS- ---------- ----KKL-YY N--INYIEQI PIP
#34 S7-16                 EPKIIS---S LSNSCNYSNN YYNNNNY--- ---------- ----KKL-YN N--INYIEQ- --P
```

Figure 6.4. Comparing the hypervariable region (HVR) amino acid sequences of representative drones sampled from the WWBBP population (black) with representative alleles obtained from geographically broad sources previously published by Hasselman and Beye, (2004) (in red). Sequences were manually aligned and an attempt was made to group similar sequences. No identical matches were found.

6.4 Discussion

Sequencing showed that the HVR fragment size variation (revealed by genotyping) is mostly due to differing combinations and numbers of (N_{1-4}) Y repeats. Substantial single nucleotide variation was also revealed by sequencing, both within and around the HVR. Comparisons between representative sequences taken from the WWBBP population and previously published data sourced from a broad geographic distribution, suggest that the local population is genetically diverse. Although a degree of diversity was expected, the extent of these population level observations are surprising.

This region (exons 6-9) of the *csd* gene is known to have elevated rates of both synonymous and non-synonymous differences (Hasselman and Beye, 2004; Cho et al., 2006). The gene as a whole has been demonstrated to have a level of polymorphism five to 10 times that of neutral regions, and functionally distinct new alleles experience positive selection. Heterozygotes have a selective advantage and low frequency functional alleles are preferentially selected. These are recognized as the two main forces driving balancing selection at the locus.

Approximately twenty different *csd* lineages were indicated by Neighbour-Joining analysis in the WWBBP sample. Clustering trends demonstrate an apparent correlation between lineage and fragment length, though due to the nature of the data (nominal and ordinal/nominal?) the relationship was not statistically tested. The relationship was not exclusive since some fragment lengths (e.g. 466 and 458) were observed to have quite different sequences and clustered into different lineages. Nevertheless, fragment size diversity does provide a general indication of lineage diversity, and possibly of *csd* allele variation since the HVR is *the* prime candidate area thought to convey allele function. Screening fragment size variation in a representative sample of diploid worker might therefore provide an indication of sex allele diversity at the population level.

A representative sample was selected from each the above lineages. The *csd*-HVR amino acid sequences were compared to each other, and to sequence information previously published by Hasselman and Beye (2004). The conserved regions on either side of the HVR repeat region were easily aligned by hand, but phylogenetic

comparisons were not meaningful due to sequence gaps and large differences within the HVR. Nevertheless, visual comparisons suggest that this population is currently rich in *csd* diversity. The above mentioned authors had sampled colonies of *A. mellifera* from geographically and genetically diverse backgrounds: Davis (CA), Berlin, Stellenbosch (South Africa), and Ribeirão Preto (Brazil). Comparable diversity was observed in our single population. These results seem surprising, but might reflect the mixed nature of and genetic heritage of British managed bees (Harpur et al., 2012). In addition, this population of bees was primarily sourced over a number of years from a reputable and knowledgeable breeder who may have intentionally designed his program to maintain a genetically diverse population of commercially viable bees.

The diverse genetic background of these bees was revealed by a Neighbour-Joining analysis of genetic diversity of coding and non-coding sequence around the HVR. I conducted the analysis with the HVR region both included and excluded, and in both cases 13 separate lineages (two or more sequences) clustered, and another seven sequences were independent. These results are again comparable to previously published work by Hasselman and Beye (2004). They identified 15 separate *csd* lineages when comparing variation within the coding region variants of the gene. Approximately 19 alleles are thought to exist worldwide (Adam 1977).

It is currently unclear how sequence difference translates into functional variation. I explored the possibility that alleles might differ in number of $(N_{1\text{-}4})Y$ repeats found within the HVR by sequencing diploid workers that were identified by genotyping to have inherited *csd* fragments of equal lengths from either parent. Adopting a rudimentary approach, I sequenced genomic DNA, and serendipitously found evidence that variation within and around the HVR might be of functional significance. The sequence data from heterozygote individuals were unreadable in eleven of twelve cases, indicating that the allelic variants inherited from either parent were significantly different. In contrast, very similar (six nucleotide differences) sequences were found in one individual, with only three non-synonymous differences detected. All were in, or around, the HVR. One of these single nucleotide mutations resulted in an amino acid (T to R) variant in the SR domain. Such regions are known to direct protein-protein interactions, and could possibly

influence the formation of allelic heterodimers. The R site variant occurs at low frequency as it was detected only in this one individual. The other two non-synonymous differences were within the HVR.

These data suggest that if the HVR is functionally relevant, then specific alleles do not need to differ in number of amino acid residues within the HVR. Differences at a small number of relevant nucleotide locations might be sufficient to release sexual development out of the default male mode, but the high number of observed fragment sizes suggests that other forces (number of repeats in conjunction with specific nucleotide differences) might also drive the specificity of alleles.

From a practical perspective, investigating sequence diversity at the *csd* revealed encouraging results. Broad potentially relevant (i.e., functionally discrete lineages) diversity was indicated by the presence of differing numbers and combinations of repeats across the HVR. In addition, an apparent relationship between genetic lineage and size was revealed. This indicates that one could possible screen for population level *csd* diversity by investigating the degree of fragment size variation across the HVR in the population. Although the specific mechanism (and hence the source DNA sequence) of *csd* function is undefined, the degree of variation indicated in this population suggests that likelihood of diploid drone formation should be low.

Chapter 7
Final Discussion

Final Discussion

One of the aims of this thesis was to assist in the design of a small breeding program, and although limited by available time, and by difficulties imposed by poor weather, the results do have value for the purpose of small scale breeders. Results relating to local queen mating success will be of interest to all beekeepers in the region. Genetic monitoring of this specific breeding population provided an indication of the amount of genetic diversity carried by the source population, and monitoring of post selection generations revealed how these genetic signatures changed through time. Modelling and simulation work predicted how this population might genetically change through time if current breeding protocols are maintained. Hopefully, these results will be of value to beekeepers hoping to establish a breeding program, and provide guidance for integrating selection and breeding into an existing honey production operation.

7.1 Mating success

I investigated queen mating success at the West Wales Bee Breeding Program (WWBBP) during the summer of 2010. Although some queens managed to mate with sufficient numbers of drones (10 was the max), mating success was on average (7) well below the accepted species mean of 13 pairings per queen. The semen from ten or so drones is required to fill a queen's spermatheca to capacity with sperm, so it appears that approximately half of the WWBBP's queens were inadequately mated and may have had a shorter effective laying lifetime as a consequence.

Nevertheless, queens did manage to mate and establish mature colonies in 75% of cases in 2010. Similar rates of success were observed in 2011 and 2012, even though weather conditions were poor during both summers, and were particularly bad in 2012. There was variance in the 2010 mating success experiment, but overall the results suggests that queens (and drones) can usually find sufficiently prolonged periods of good mating weather. Since there is a heritable component to mating success, it might be targeted by breeding from locally strong colonies headed by second year or third year (long-lived) queens. Wales is located towards the northern limit of the honeybee's natural distribution and can experience periods of cold wet weather at any time of the year, and the WWBBP would benefit from bees that possess the qualities needed to adequately mate under marginal conditions.

7.2 Monitoring

7.2.1 Varroa

The WWBBP experienced substantial *varroa* attributed losses over the 2010/11 winter. Infestation levels were subsequently monitored in newly established colonies during 2011 and 2012, and a significant increase in mean number of mites was observed across years. Counts were taken to monitor population-wide seasonal trends, but also as an indicator of possible tolerance in young colonies. But since colony mite count is only marginally heritable, and these colonies were yet to fully mature, the program could benefit from a more rigorous screen. Recently recommended approaches that might lead to more rapid improvement in tolerance include testing for above average expression of hygienic behaviour, as well as identifying colonies with low mite reproduction rates (Fries, 2012). Screening for hygienic behaviour is demanding of both time and effort (and must be repeated for consistent results) for the small scale commercial operator to seriously consider. The latter of these two tests (i.e., lower mite reproduction rates) would provide a better benefit return, and is simpler and most practical.

Programs intent on directing population adaptation through purposeful selection should consider screening colonies with second year queens for low rates of mite population growth. The methodology accounts for differences in initial infestation between colonies and are applied to mature colonies with actively laying queens only. Early and late season counts are needed, so that the rate of mite reproduction can be determined (Büchler et al., 2010; Lee 2010; Fries, 2012). Following recommendations by Büchler et al. (2010), the first data point is acquired by counting the natural mite drop (number of mites falling out of the hive) over the first 3-4 weeks of brood production in the spring (standardized by being carried out during the *Salix* bloom). The second data point demands a little more effort, and is acquired by counting the number of mites infecting a sample of 300 bees taken from the honey combs in the uppermost box. Since *varroa* reproduce in honeybee brood cells, counts are usually determined by taking nurse bees off the brood nest Fries (2012), a potentially time consuming operation since the queen must first be located and secured. However, bees from a honey-box are thought to have a more uniform infestation (Büchler et al., 2010) and can be sampled without having to access the

brood and locating the queen. Relatively inexperienced but competent personnel can perform *varroa* counts this way. Nevertheless, if counts are taken at strategically convenient times (e.g., before and after the early summer nectar flows) the approach should be considered by suitably resourced programs.

7.2.2 Locating the Queen

Locating queens (the hardest part of sampling from the brood-nest) is much easier if they are marked, but most commercial operation do not go to the trouble of marking queens destined for production colonies. Young mated queens are easier to find in small colonies, and can most conveniently be marked during the transition from the smaller mating hives into full sized colony box. Mature colonies that have experienced a supercedure or swarming events, both performance relevant data, are easier to identify if the queen is marked and any new (unmarked) queens must be marked for the system to practical in the long term. Clearly, such work is an added management burden for beekeepers, and an efficient record keeping program must be implemented (and maintained) for maximum benefit. Many European countries offer small scale breeders federal assistance and support, but this level of organization is not currently available in the UK. Small scale breeding programs need to be self-sufficient and allocate their own resources accordingly.

7.2.3 Production and colony strength

Colony strength was estimated by visual assessment using a standardized approach. Although this method provided only approximate estimates, it allowed colony strength to be efficiently assessed during a single monitoring event. Digital methods for assessing the amount of brood on a frame have been developed. These were considered, but were rejected for the following reasons. It was recommended that all bees should be shaken off brood-frames for digitalized programs to work. Such treatment was considered too disruptive and not conducive to colony wellbeing. Nevertheless, attempts were made to photograph frames in the field with bees still attached. Attempts were made to standardize methodology, but this proved difficult in the field setting where colonies were spread out across the moor on wooden pallets. Studies utilizing digital assessments have used camera tripods and frame holders at fixed distances (on level ground) to standardize image quality between frames. Each monitoring sweep would also require approximately 720 images, or 12 images per

colony on the heather. The visual method was adopted after a frank cost benefit analysis.

Colonies of known size gained weight at different rates as they foraged on heather nectar and pollen in 2011. Conditions allowed for the foraging efficiency of bees from different colonies to be compared. Analysis revealed no statistical correlation between colony size (frames of bees) and weight-gained during a nectar flow that year. These results suggested that there was variance in foraging efficiency, possibly due to genetic (hence heritable) variation in the population. Conditions were poor on the heather in 2012 and did not allow for such comparisons. Colonies gained weight initially (partly due to increased number of hatching adults bees) but colony weights declined after all available nectar flows ceased. Nevertheless, a number of choice colonies stood out due to superior size and weight at the end of the season. There was a statistically significant reduction in the variance of colony size (frames of bees and amount of brood) between 2011 and 2012 daughter colonies. The 2012 colonies were also visibly uniform on inspection, with consistent layout of brood nest and stores. This increased uniformity is likely due to the selective pressures being applied across generations.

7.3 Genetic monitoring and modelling

Genetic monitoring revealed ample variation at both neutral microsatellite loci, and within the hyper-variable region of the *csd* in the source population. Broader genome-wide variation is not necessarily inferred by these results since correlations between phenotypic variation, and variation observed at a small number microsatellite, markers are generally weak. Nevertheless, these observations do suggest that the Tropical Forest source population might contain adaptive potential, and should be responsive to selective breeding applied by the WWBBP.

Monitoring also revealed that low frequency alleles were being lost due to chance, with significant differences in allelic richness being observed between the source population and subsequent generations. There was also a general consensus among various effective population size estimators indicating a slight declines in population size was occurring with each round of breeding. Loss of genetic diversity is an inevitable consequence of closed population line breeding, but it must be limited

since adaptive selection is less effective when diversity low. Maintaining population level diversity will provide fitness benefits at the colony level since it has been demonstrated that genetically diverse colonies are more resistant to environmental and disease related perturbations. It is also known that a genetically diverse work-force can maintain a more balanced nest environment than a genetically limited workforce (Jones et al., 2004).

7.4 Breeding

Within family selection has been recommended for programs that can only maintain a limited number of queen lines, and is the approach currently adopted by the WWBBP (Moritz, 1986). Approximately 150 colonies are currently maintained and it is proposed that new colony production will be limited to approximately 60 queens from 4 breeders each year (12-20 daughter queens per line). My *csd* model suggested that a population of this effective size can only maintain 5 to 6 sex (*csd*) alleles, and diploid drone production could increase before stabilizing at 17-18% per colony, and it is possible (depending on the current number and frequency of sex alleles in the population) that the mean colony brood viability could drop to 85% within 5 years.

It is unlikely that the WWBBP's bee population will suffer from the effects of genetic depletion, at least in the short term, but indications are that diversity could become limiting if current program protocols are maintained. This population might therefore reach its maximum improvement potential within a relatively short time frame, and program managers might consider increasing the number of queen lines it maintains in order to maintain adaptive potential within the population. This might be achieved by staggering queen lines, so that each line is only bred from every other year. By staggering this way, it is probably feasible for programs the size of the WWBBP to maintain eight queen lines in total. Four queen breeders (one each from a different line) could be selected one year, and the best colony from each of the other four lines the following year. Staggering breeding lines might allow genetic diversity could be maintained, and daughter colonies can be monitored for two full seasons (and winters) before becoming eligible for breeder selection. One disadvantage of this approach might be that uncontrolled environmental effects could complicate colony comparisons. All non-breeder colonies will be part of the production population, and

will be located in different apiaries and exposed to differing environmental conditions.

There was a statistically significant difference in allelic richness between the source population and both the 2011 and 2012 daughter populations, but not between the two daughter (2011 and 2012) populations. It is possible that genetic differentiation may have been is dampened by a potentially broader drone contribution in 2012. Two daughter colony groups were raised in 2012 and each was mated at different locations and exposed to drones of different sources. Such an approach could possibly be adopted to limit potential genetic depletion due limiting number of breeders. However, it would be worth considering the origin of the male contribution as there is evidence that the difference in drone input could have influenced the temperament of the 2012 bees.

Bee mating behaviour is inherently uncertain since queens must pair on the wing with multiple drones (of potentially unknown origin) some distance from the nest. The process is assumed to be random as little is known about queen mate choice, though drones from disease resistant colonies might have a breeding advantage due to better conditioning. Breeders can influence the transfer of desirable traits via male lines by flooding mating areas with drones mothered by daughters of colonies displaying the characteristics of choice. The process remains vulnerable to the vagaries of the weather, and ultimately, breeders have little control over the number or the origin of the drones their selected queens mate with.

Contrastingly, breeders have complete control over the number and origin of the queen lines they select, and the number of daughter colonies they establish each year. Modelling showed that the number of breeders has the most influence over the change in allele frequency variance, and the change in population genetic diversity across generations. For breeders employing open mating, breeder selection is the key component affecting progressive adaptation.

The WWBBP used eight effective breeders, selected from a source population of approximately 150 colonies in 2011. Approximately 100 daughter colonies were raised that summer, and the top 30 performing colonies (based on foraging ability, *varroa* infestation and temperament), including representatives from each queen line, were highlighted as potential breeding stock. Four breeders, each from a different

queen line, were selected from this stock the following year and 60 or so new colonies established. Not all breeders had equal representation in the daughter population, which comprised of two groups grafted from the same four breeders. These two daughter cohorts were openly mated at different locations and were subjected to differing drone mediated influences. No significant genetic differentiation was detected between the colonies mated at different locations in 2012; hence they were pooled for analysis. There was, however, an apparent difference in the temper of the bees mated at the two locations. The behavioral difference could possibly be attributed to the expression of different drone-mediated defense alleles.

7.5 Considerations for breeders

Declining honeybee health has made commercial beekeeping an increasingly labour intensive occupation. Selectively breeding for local adaptation can be part of the solution since it can enhance, at the population level, those heritable characteristics that allow colonies to be productive and disease tolerant under local conditions, thus reducing the need for supplemental feeding and medication. However, breeding protocols must be simple to be of practical use to small-scale breeders, and should provide a timely return (improvement in the population within a few generations) for invested effort. Breeders must concurrently guard against depleting genetic diversity, and the resulting detrimental expression of inbreeding effects (such as diploid drone production). Integrating the logistics of a breeding program into an already busy honey-production schedule is part of the challenge, and these are further complicated when production colonies are migrated (to the heather for example). The challenge of this project was to enhance local suitability while concurrently preserving genetic diversity and adaptive potential in the simplest most practical way. A major goal of this project was to develop a transferable template for successful small-scale breeding. Recognizing that individual businesses must be managed independently, certain considerations should be universally addressed by all seriously considering selective breeding. Amount of effort and focus directed to any one will depend on the specific circumstances.

7.5.*1 Consideration for small scale breeders*

a) ***Tracking colony/queen performance.*** *Traditionally done using a paper spreadsheet or sometimes relevant information is written directly onto a hive label. Data is often transferred onto a computer based system at a later date. Online applications are becoming increasingly available (Beetight, Hivetrack), and some offer online access to a personal database via a cellphone application. Whatever the adopted approach, the process needs to be simple and practical in the field. Regardless, one needs an organized and disciplined approach. Possibly allocate days for assessing colony performances only as relevant data can easily go unrecorded during the hustle and bustle of a regular beekeeping day (e.g. while splitting colonies, removing honey-crops). This might not be possible as out-yards are frequently widely distributed, and not frequently visited. The adopted method will depend in part upon the resources available, and on management style.*

b) *Marking queens helps to identify colonies that have recently experienced a supercedure or swarming event. Un-marked queens are easier to find in when colonies are small. Young mated/laying queens could be most conveniently marked sometime before her colony expands to full size.*

c) *Colonies/queen performance should preferably be monitored for a minimum of 2 years (survive 2 winters) before being considered for breeding stock. If selecting for mite resistance, then monitoring should compare mite reproduction rates among established colonies (probably during the second season). Mite numbers can still be knocked down with a soft treatment ((e.g.3.5% oxalic acid) if loads threaten overwintering survival (generally over 10% infestation), without affecting rate of mite population growth during the summer season. If resources are limited (e.g. lack of skilled personnel) and monitoring impractical (same applies in part to a), then select for tolerance by selecting the healthiest best performing colonies.*

d) *Genetically depleted populations do not effectively adapt in response to selective breeding. Protect against genetic depletion by breeding from multiple queen lines. Models suggest that a closed population can be maintained with minimal loss if 12 breeders are used and a 100 or so new colonies raised each year. Smaller outfits could probably maintain adequate long term diversity with 8 breeders, as long as each was from a different queen-line (and possibly stagger so that each line is only used every other year). Diploid drone production is also more likely to occur in populations lacking genetic diversity and mean brood viability may drop to unacceptable levels if too many sex alleles are lost due to chance. Effective population size depends on the number of breeders (male and female) contributing to the next generation, and it in turn affects the number of sex-alleles the population can maintain.*

e) *Breeders have greater 'control' over drone contribution when mating yards are isolated. One approach might be to use the daughter colonies of the previous year's breeders as drone mothers. This way the breeding characteristics of the selected 'grandmother' colonies will be propagated through the male (grandson) line.*

f) *Account for environmental effects by dispersing colonies from each queen specific line into different locations.*

7.6 A final thought

Small-scale breeding programs are handicapped by scale. Few can afford to sacrifice the time and labour, or have the resources necessary to produce and monitor (performing *varroa* counts for example) large numbers of colonies. Collaborative efforts have proven successful, where the burden of maintaining sufficient numbers of queen lines to offset potential inbreeding effects, and raising sufficient numbers of new daughter colonies per line to expose desired (and undesired) adaptive characteristics, is shared. Independent small-scale breeders must also maintain multiple queen lines to offset inbreeding, but each line will contribute relatively few daughters (to the next generation) in comparison to that of large scale programs, and adaptive 'progress' will be slower. Key recommendations include:

- Pick as many of your best colonies as you can for breeding, and raise as many queens from each as possible to capture as much adaptive potential as possible.
- Use all these daughter colonies as drone produces the following year and consider screening for low rates of mite reproduction.
- To increase number of queen lines maintained, consider breeding from best line colony each every other year
- Don't let temperamental colonies breed (re-queen if you can), or be drone sources.

7.7 Further work

The honeybee genome sequence was published in 2006 (Whitfield et al., 2006). Since then, genome level sequencing and screening technologies have continued to advance. Geneticists now have available powerful tools to investigate genome level variation among groups of individuals, and these advances offer beneficial opportunities for breeding. For example, being able to associate specific genetic signatures (markers) with particular behaviours (phenotypes) would aid marker assisted selection; that is the selection of breeding individuals based on identifiable genetic characteristics. Recent research has shown that the honeybee has a small genome and a high recombination rate, properties that make the use of quantitative trait loci (QTL's) particularly suitable for detecting genomic regions with behavioral significance and influence.

Some progress has been made. QTL's that influence hygienic and defensive behaviour are now available, though none of this knowledge has yet had much practical implication. Finding QTL's or genes influencing other colony level traits such as honey production and swarming will require additional advanced molecular and statistical work, as well as large sample sizes to confirm results. It would require the combined expertise of a well-funded and equipped genetics facility along with a proficient and sizable beekeeping program with demonstrable variance in the trait of interest within its bee population. Assisted marker selection in honeybees is still an emerging field, and our understanding of the process rudimentary. Finding genetic locations correlating with trait-specific significance would be a start, but much work would still remain to understand how to effectively proliferate the desired character through crossbreeding.

Since drone 'performance' has little or no tangible influence on desired colony traits, they have historically received little attention by breeders. Nevertheless, the haploid state of drones (males) could aid selection in honeybees since it allows for the direct testing of individual level traits. For example, selecting drones expressing notable disease (e.g. virus) resistance could help improve tolerance at the colony level. Testing traits at the colony level is more complicated since queen honeybees mate with multiple males and the observed characteristics can result from interactions between a complex mix of genetic backgrounds. This has been a major barrier to breeding improvement in bees. Consequently, little progress utilizing available genomic level information of practical significance has yet been made.

Agricultural practices have become increasingly mechanized across the developed world over recent decades. These developments have challenged honeybees due to loss of suitably diverse forage, and increased pesticide exposure. Worldwide research and debate continue about the possible detrimental influences of pesticides on honeybee health and productivity, and concern is frequently expressed about the environment in general. From a regional perspective, the topographical nature of the landscape in North and mid-Wales has limited industrialization. The region could therefore be a viable control area (in relation to other areas where more mechanized forms of farming are practiced) for experimental work designed to investigate how pesticides affect (e.g. neonicotinoids) bees under field conditions.

The distribution of *Apis mellifera* expanded in concert with human expansion out of Europe during the latter centuries of the last millennium. They thrived in many areas and colonies can now be found globally within a belt that extends approximately 60° north and south of the equator. Bees became crucial for the production of economically important commercial agricultural crops in many regions. However, as the recent declines due to *varroa* and associated viruses demonstrate, bees can be susceptible to novel pest and disease threats. Because of this, regulations have periodically been implemented to limit the movement of bees across international borders. The United States has imposed a decade's long ban restricting the importation of bees from Europe in response to the catastrophic losses observed in Britain during the early part of the century. Bees can currently be imported from Canada and New Zealand only. Australia has restricted imports from all sources since 2008, but ten choice queens were recently allowed entry from Canada under strict quarantine (Thistelton, 2013). These queens were sought in efforts to boost disease resistance and vitality in isolated populations of Australian bees. Similar concerns have been expressed concerning the limited genetic resources (due to historical import restrictions) available in North America. In response, fresh genetic stocks (queens and sperm of A .m. caucasica) have recently been sourced under quarantine from Turkey and Georgia (Sheppard, 2013).

My studies revealed high levels of genetic and phenotypic variation (possibly due in part to a mixed genetic heritage) within a small managed population in Wales. By characterizing and isolating lines with specific colony-level traits, (e.g. good temperament, production, frugality etc.) this population could provide a genetic reservoir for other regions in Britain. A broader geographic perspective could also be considered. Although the importation of live bees from Britain into many countries is restricted (and likely to remain so) the movement of germplasm (sperm and eggs) across international borders is possible (Hopkins et al., 2012), and permitted with certain precautions. The transportation of fertilized eggs is probably preferable since they are far easier to collect and carry a complete (male and female) genetic package. Queens can then be raised from eggs selected from chosen colonies at destination. Small scale European bee-breeding operations (such the WWBBP) could be genetic reservoirs for global regions suffering declining production and health due to genetic depletion.

Bee breeding could also benefit if we understood how separate *csd* alleles differ. It is known that region 3 *csd* sequences consistently cluster into separate lineages, and each cluster is currently considered to be a functionally distinct allele class. Using this approach, Hasselmann et al., (2008) and Yong Liu et al., (2012) report that putatively neutral variants from the same class of *csd* sequences had the same repeat structure in the HVR. Individuals carrying alleles from the same class should therefore develop into diploid drones. Controlled mating experiments could provide insight into the appropriateness of this classification measure. For example, by utilizing instrumental insemination (II), virgin queen of known *csd* lineage could be singly mated with sperm taken from a drone carrying a functionally similar *csd* allele. The matching of functionally identical alleles would result in a 50% brood viability since half the brood would develop as diploid drones and would be removed by the colony worker-force. With time, a concrete picture illustrating of how separate alleles are functionally related could be constructed.

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Appendix i: Table A1. 2011 colony data

QueenID	QueenID	Date of Birth	Nuc to hive transfer date	Colony Strength	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Date	Varroa Count	Frames w bees	Stores	Temperament	Score	Queen/brood/disease
312	Anwen 1.1	21.6.11	22.7.11	18/+	16.8.11	13.24	26.48	21.8.11	15.10	30.20	23.8.11	18.18	36.36	29.8.11	19.65	39.30	30.9.11	12.76	25.52	01.10.11	1	9	H+	Ok	3	
327	Anwen 1.2	21.6.11	22.7.11	18/0	16.8.11	15.84	31.68	21.8.11	18.72	37.44	23.8.11	22.94	45.88	29.8.11	24.20	48.40	30.9.11	15.75	31.5	01.10.11	6	9	H	Calm	2	
317	Anwen 1.3	21.6.11	22.7.11	18/+	16.8.11	14.00	28.00	21.8.11	16.02	32.04	23.8.11	18.08	36.16	29.8.11	20.45	40.90	30.9.11	14.78	29.56	01.10.11	3	8	P and H	Ok	3	
320	Anwen 1.4	21.6.11	22.7.11	18/0	16.8.11	12.77	25.54	21.8.11	13.00	26.00	23.8.11	16.08	32.16	29.8.11	17.45	34.90	30.9.11	12.7	25.4	01.10.11	2	5	No stores	Ok	3	MYC mutt
316	Anwen 1.5	21.6.11	22.7.11	13/0	16.8.11	14.64	29.28	21.8.11	15.67	31.34	23.8.11	19.90	39.80	29.8.11	21.78	43.56	30.9.11	13.54	27.08	01.10.11	4	8	No stores	Ok	3	MYC mutt
324	Anwen 1.6	21.6.11	22.7.11	15/0	16.8.11	13.02	26.04	21.8.11	20.30	40.60	23.8.11	23.72	47.44	29.8.11	24.47	48.94	30.9.11	17.36	34.72	01.10.11						
319	Catrin 1.3	21.6.11	22.7.11	18/0	16.8.11	16.24	32.48	21.8.11	18.30	36.60	23.8.11	22.02	44.04	29.8.11	24.04	48.08	30.9.11	17.24	34.48	01.10.11	0		P + H	Ok	3	
325	Catrin 1.4	21.6.11	22.7.11	21/+	16.8.11	13.56	27.12	21.8.11	16.14	32.28	23.8.11	22.04	44.08	29.8.11	23.80	47.60	30.9.11	16.52	33.04	01.10.11	1	8	H + P	Ok	3	Darker
332	Catrin 1.5	21.6.11	22.7.11	10/o	16.8.11	14.38	28.76	21.8.11	16.28	32.56	23.8.11	19.68	39.36	29.8.11	21.43	42.86	30.9.11	15.24	30.48	01.10.11	2	5	H and P	Ok	3	Darker
331	Catrin 1.6	21.6.11	22.7.11	7/o	16.8.11	15.86	31.72	21.8.11	16.93	33.86	23.8.11	20.66	41.32	29.8.11	20.83	41.66	30.9.11	15.7	31.4	01.10.11	5	8	H P++	Irritated	2	Bigger queen
330	Catrin 1.7	21.6.11	22.7.11	17/0	16.8.11	15.22	30.44	21.8.11	18.20	36.40	23.8.11	19.30	38.60	29.8.11	21.78	43.56	30.9.11	21.08	42.16	01.10.11	1	?	H++	Ok	3	MYC mutt
328	Catrin 1.8	21.6.11	22.7.11	15/-	16.8.11	14.26	28.52	21.8.11	16.20	32.40	23.8.11	19.66	39.32	29.8.11	21.35	42.70	30.9.11	14.76	29.52	01.10.11	0	8	H and P	Ok	3	MYC mutt
322	Catrin 1.9	21.6.11	22.7.11	19/+	16.8.11	16.40	32.80	21.8.11	18.71	37.42	23.8.11	23.06	46.12	29.8.11	24.56	49.12	30.9.11	17.14	34.28	01.10.11	4	9	H and P	Ok	3	??
329	Catrin 1.10	21.6.11	22.7.11	17/0	16.8.11	13.02	26.04	21.8.11	15.20	30.40	23.8.11	18.28	36.56	29.8.11	19.50	39.00	30.9.11	13.5	27	01.10.11	1					Darker
326	Catrin 1.11	21.6.11	22.7.11	11/-	16.8.11	13.32	26.64	21.8.11	13.30	26.60	23.8.11	13.88	27.76	29.8.11	15.17	30.34	30.9.11	14.01	28.02	01.10.11	4	6	H and P	Ok	3	MYC mutt
318	Catrin 1.15	25.6.11	22.7.11	11/o	16.8.11	15.20	30.40	21.8.11	16.22	32.44	23.8.11	19.94	39.88	29.8.11	21.56	43.12	30.9.11	15.84	31.68	01.10.11	6	??	??	V. defensive	1	??
311	Catrin 1.16	25.6.11	22.7.11	14/+	16.8.11	19.76	39.52	21.8.11	16.93	33.86	23.8.11	21.56	43.12	29.8.11	23.32	46.64	30.9.11	15.41	30.82	01.10.11	5	6	H and P	Ok	3	Light with black tip
313	Branwen 1.1	25.6.11	10.8.11	12/o	16.8.11	12.70	25.40	21.8.11	13.22	26.44	23.8.11	18.12	36.24	29.8.11	19.65	39.30	30.9.11	13.4	26.8	01.10.11	6	6	H and P	Ok	3	Beautifull
289	Branwen 1.3	25.6.11	10.8.11	???	16.8.11	12.38	24.76	21.8.11	12.42	24.84	23.8.11	11.28	22.56	29.8.11	12.76	25.52	30.9.11	11.84	23.68	01.10.11	2	5	H and P	Ok	3	Nice darker
315	Branwen 1.4	25.6.11	10.8.11	12/o	16.8.11	12.34	24.68	21.8.11	13.96	27.92	23.8.11	16.72	33.44	29.8.11	18.18	36.36	30.9.11	13.78	27.56	01.10.11	4	5	H and P and Brood	Ok	3	MYC mutt
309	Branwen 1.5	25.6.11	22.7.11	11/o	16.8.11	12.10	24.20	21.8.11	13.02	26.04	23.8.11	17.34	34.68	29.8.11	19.40	38.80	30.9.11	13.7	27.4	01.10.11	4	5	H and P	Ok	3	MYC mutt
266	Carys 1.3	1.7.11	10.8.11	17/+	16.8.11	15.56	31.12	21.8.11	16.88	33.76	23.18.11	20.06	40.12	23.18.11	22.80	45.60	30.9.11	17.32	34.64	01.10.11	6	?	H and P	ok	3	Smallish Dark
261	Carys 1.4	1.7.11	10.8.11	13/+	16.8.11	11.90	23.80	21.8.11	12.74	25.48	23.8.11	13.20	26.40	29.8.11	13.87	27.74	30.9.11	12.72	25.44	01.10.11						
276	Carys 1.5	1.7.11	10.8.11	23/+	16.8.11	12.88	25.76	21.8.11	14.32	28.64	23.8.11	18.00	36.00	29.8.11	19.55	39.10	30.9.11	15.1	30.2	01.10.11	3	8	H and P	Very V. calm	5	Dark queen
271	Carys 1.6	1.7.11	10.8.11	20/+	16.8.11	16.66	33.32	21.8.11	18.22	36.44	23.8.11	23.12	46.24	29.8.11	25.40	50.80	30.9.11	19.66	39.32	01.10.11	2			Irritated	2	
279	Carys 1.10	1.7.11	10.8.11	11/+	16.8.11	11.50	23.00	21.8.11	12.18	24.36	23.8.11	14.70	29.40	29.8.11	16.24	32.48	30.9.11	14	28	01.10.11	4	7	H and P	Ok	3	Darker
264	Carys 1.11	1.7.11	10.8.11	13/++	16.8.11	14.22	28.44	21.8.11	15.22	30.44	23.8.11	19.10	38.20	29.8.11	21.60	43.20	30.9.11	13.95	27.9	01.10.11	1	7	H and P	very . Calm	4	Mutt
270	Carys 1.12	1.7.11	10.8.11	11/+	16.8.11	12.86	25.72	21.8.11	13.48	26.96	23.8.11	16.86	33.72	29.8.11	18.70	37.40	30.9.11	13.18	26.36	01.10.11	1			Irritated	2	
275	Carys 1.13	1.7.11	10.8.11	6+ lots bs	16.8.11	16.36	32.72	21.8.11	16.12	32.24	23.8.11	14.38	28.76	29.8.11	15.69	31.38	30.9.11	15.4	30.8	01.10.11	5	6	H+	Ok	3	

Appendix i continued. 2011 Colony data

QueenID	QueenID	Date of Birth	Nuc to hive transfer date	Colony Strength	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Date	Varroa Count	Frames w bees	Stores	Temperament	Score
282	Llinos 1.1	30.6.11	10.8.11	19/+	16.8.11	12.38	24.76	21.8.11	13.08	26.16	23.8.11	16.20	32.40	29.8.11	17.50	35.00	30.9.11	11.88	23.76	01.10.11	13	4	H and P	ok	3
281	Llinos 1.2	30.6.11	10.8.11	21/+	16.8.11	14.02	28.04	21.8.11	15.24	30.48	23.8.11	18.06	36.12	29.8.11	20.11	40.22	30.9.11	15.86	31.72	01.10.11	0	9	H and P	Ok	3
310	Llinos 1.3	30.6.11	10.8.11	8/-	16.8.11	12.90	25.80	21.8.11	13.66	27.32	23.8.11	11.96	23.92	29.8.11	12.72	25.44	30.9.11	11.74	23.48	01.10.11	7	5	Small amount of H	ok	3
283	Llinos 1.5	30.6.11	10.8.11	15/0	16.8.11	12.90	25.80	21.8.11	13.36	26.72	23.8.11	15.60	31.20	29.8.11	17.45	34.90	30.9.11	13.46	26.92	01.10.11	6	6	Some brood, H-	ok	3
285	Llinos 1.6	30.6.11	10.8.11	15/++	16.8.11	14.94	29.88	21.8.11	16.60	33.20	23.8.11	19.28	38.56	29.8.11	20.80	41.60	30.9.11	16.68	33.36	01.10.11	6		H + and P+	Ok	3
280	Llinos 1.7	30.6.11	10.8.11	15/+	16.8.11	14.78	29.56	21.8.11	16.00	32.00	23.8.11	19.14	38.28	29.8.11	19.68	39.36	30.9.11	15.4	30.8	01.10.11	4		H+	Irritated	2
284	Llinos 1.10	30.6.11	10.8.11	8/o	16.8.11	10.14	20.28	21.8.11	11.60	23.20	23.8.11	11.54	23.08	29.8.11	12.30	24.60	30.9.11	11.16	22.32	01.10.11	3	6	Some H and P	Ok	3
286	Llinos 1.11	30.6.11	10.8.11	8/o	16.8.11	12.66	25.32	21.8.11	12.04	24.08	23.8.11	12.48	24.96	29.8.11	13.58	27.16	30.9.11	12.86	25.72	01.10.11	?	small		Ok	3
297	Llinos 1.12	30.6.11	10.8.11	11/+	16.8.11	11.54	23.08	21.8.11	13.58	27.16	23.8.11	18.48	36.96	29.8.11	20.20	40.40	30.9.11	14.6	29.2	01.10.11	4	6	H+	Ok	3
287	Marged 1.6		10.8.11	13/0	16.8.11	12.02	24.04	21.8.11	12.74	25.48	23.8.11	16.84	33.68	29.8.11	22.18	44.36	30.9.11	16.3	32.6	01.10.11	?		small amount of H	Ok	3
298	Nia 1.1	07/01/2011	10.8.11	8/+	16.8.11	14.12	28.24	21.8.11	14.8	29.60	23.8.11	17.52	35.04	29.8.11	19.35	38.70	30.9.11	14.66	29.32	01.10.11					
290	Nia 1.3	07/01/2011	10.8.11	9/+	16.8.11	14.1	28.20	21.8.11	14.02	28.04	23.8.11	17.56	35.12	29.8.11	18.78	37.56	30.9.11	13.94	27.88	01.10.11					
300	Nia 1.5	07/01/2011	10.8.11	15/+	16.8.11	11.14	22.28	21.8.11	12.64	25.28	23.8.11	17.2	34.40	29.8.11	21.07	42.14	30.9.11	14.26	28.52	01.10.11	1			V.V.defensive	1
294	Nia 1.12	07/01/2011	10.8.11	15/+	16.8.11	13.06	26.12	21.8.11	13.32	26.64	23.8.11	19.66	39.32	29.8.11	17.28	34.56	30.9.11	12.96	25.92	01.10.11				V.V.defensive	1
291	Nia 1.13	07/01/2011	10.8.11	21/+	16.8.11	12.9	25.80	21.8.11	14.12	28.24	23.8.11	17.24	34.48	29.8.11	19.74	39.48	30.9.11	13.28	26.56	01.10.11					
292	Nia 1.14	07/01/2011	10.8.11	15/+	16.8.11	11.22	22.44	21.8.11	12.88	25.76	23.8.11	17.14	34.28	29.8.11	19.34	38.68	30.9.11	12.88	25.76	01.10.11	3			V. defensive	2
295	Nia 1.15	07/01/2011	10.8.11	7/o	16.8.11	14.66	29.32	21.8.11	13.65	27.30	23.8.11	11.78	23.56	29.8.11	12.29	24.58	30.9.11	11.78	23.56	01.10.11	?		Very weak	ok	3
303	Nia 1.17	07/01/2011	10.8.11	11/+	16.8.11	13.04	26.08	21.8.11	13.2	26.40	23.8.11	14.7	29.40	29.8.11	16.04	32.08	30.9.11	??		01.10.11	2	6	H+	Ok	3
323	Nia 1.18	07/01/2011	10.8.11	14/+	16.8.11	16.68	33.36	21.8.11	16.74	33.48	23.8.11	19.68	39.36	29.8.11	20.5	41.00	30.9.11	14.8	29.6	01.10.11	10	7	H and P	V. defensive	2
314	Nia 1.20	07/01/2011	10.8.11	8/+	16.8.11	14.34	28.68	21.8.11	14.1	28.20	23.8.11	17.32	34.64	29.8.11	18.1	36.20	30.9.11	12.32	24.64	01.10.11					
518	Gwenllian 1.8	07/08/2011	17.8.11	17/0				21.8.11	11.96	23.92	23.8.11	15.81	31.62	29.8.11	17.05	34.10	30.9.11	12.68	25.36	01.10.11				V, Defensive	2
267	Gwenllian 1.10	07/08/2011	17.8.11	17/+				21.8.11	12.46	24.92	23.8.11	15.56	31.12	29.8.11	17.7	35.40	30.9.11	13.16	26.32	01.10.11	5	4	H and P	Ok	3
515	Lucy29 1.1	07/08/2011	17.8.11	21/+		Not		21.8.11	13.24	26.48	23.8.11	18.06	36.12	29.8.11	19.87	39.74	30.9.11	15.16	30.32	01.10.11	1	6	H and P	ok	3
511	Lucy29 1.2	07/08/2011	17.8.11	19/++				21.8.11	14.02	28.04	23.8.11	20.26	40.52	29.8.11	21.3	42.60	30.9.11	14.8	29.6	01.10.11	50	6	H and P	ok	3
259	Lucy29 1.3	07/08/2011	17.8.11	19/0		on		21.8.11	17.4	34.80	23.8.11	19.74	39.48	29.8.11	21.5	43.00	30.9.11	16.68	33.36	01.10.11	8	6	H and P	Ok	3
272	Lucy29 1.6	07/08/2011	17.8.11	19/0				21.8.11	13.7	27.40	23.8.11	18.66	37.32	29.8.11	21.2	42.40	30.9.11	14.8	29.6	01.10.11	10	5	H and P	ok	3
269	Lucy29 1.8	07/08/2011	17.8.11	16/+		Heather		21.8.11	14.84	29.68	23.8.11	21.6	43.20	29.8.11	21.4	42.80	30.9.11	13.82	27.64	01.10.11	2	5	H++ and p	Defensive	2
273	Lucy29 1.9	07/08/2011	17.8.11	20/+				21.8.11	13.16	26.32	23.8.11	16.36	32.72	29.8.11	17.2	34.40	30.9.11	13.1	26.2	01.10.11	5			Defensive	2
274	Lucy29 1.13	07/08/2011	17.8.11	19/0				21.8.11	15.84	31.68	23.8.11	19.02	38.04	29.8.11	20.67	41.34	30.9.11	16.48	32.96	01.10.11	1	6	H and P	ok	3
260	Dwynwen 1.6	07/08/2011	17.8.11	10/+		yet		21.8.11	12.84	25.68	23.8.11	12.18	24.36	29.8.11	12.7	25.40	30.9.11	11.64	23.28	01.10.11	8	5	H and p	ok	3
277	Dwynwen 1.9	07/08/2011	17.8.11	13/0				21.8.11	11.16	22.32	23.8.11	12.1	24.20	29.8.11	13.64	27.28	30.9.11	13.1	26.2	01.10.11	13	4	H and P	ok	3
263	Sioned 1.1	07/08/2011	17.8.11	11/+				21.8.11	14.46	28.92	23.8.11	18.26	36.52	29.8.11	19.22	38.44	30.9.11	14.08	28.16	01.10.11	0	5	SOME STORES	ok	3

Appendix ii. Table A2. 2012 colony data

MotherID	ColonyID	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Weight change 26/8-6/9	Date	Weight Measured	Hive Weight Kg	Date	Varroa Count	Frames w bees	Frames w brood	brood	Temperament LLandegla 4/9/12
Anwen 2.1	1	08/08/2012	13.72	27.44	26/08/2012	16.28	32.56	06/09/2012	17.30	34.60	2.04	08/09/2012	16.74	33.48	08/09/2012	11	6	3	10	15
Anwen 2.2	369	08/08/2012	14.78	29.56	26/08/2012	16.90	33.80	06/09/2012	16.12	32.24	-1.56	08/09/2012	16.09	32.18	28/08/2012	2	7	4	15	2
Anwen 2.3	287	08/08/2012	12.74	25.48	26/08/2012	15.30	30.60	06/09/2012	18.78	37.56	6.96	08/09/2012	16.52	33.04	28/08/2012	19	6	3	13	15
Anwen 2.4	9	08/08/2012	17.02	34.04	26/08/2012	19.08	38.16	06/09/2012	19.98	39.96	1.80	08/09/2012	19.28	38.56	28/08/2012		8	3.5		
Anwen 2.5	419	08/08/2012	16.48	32.96	26/08/2012	18.04	36.08	06/09/2012	20.68	41.36	5.28	08/09/2012	20.98	41.96	28/08/2012	10	6	4	11	16
Anwen 2.6	13	08/08/2012	15.30	30.60	26/08/2012	18.04	36.08	06/09/2012	18.60	37.20	1.12	08/09/2012	18.24	36.48	28/08/2012	5	6	2	9	1
Anwen 2.7	26	08/08/2012	18.50	37.00	26/08/2012	19.90	39.80	06/09/2012	20.76	41.52	1.72	08/09/2012	20.42	40.84	02/09/2012					22
Anwen 2.8	47				26/08/2012	16.50	33.00	06/09/2012	16.50	33.00	0.00					30	6	3	13	0
Anwen 2.9	48				26/08/2012	18.60	37.20	06/09/2012	18.48	36.96	-0.24					1	6	4		0
Anwen 2.10	49				26/08/2012	17.50	35.00	06/09/2012	17.50	35.00	0.00					9	6	3		0
Carys 2.1	2	08/08/2012	22.30	44.60	26/08/2012	17.88	35.76	06/09/2012	9.52	19.04	-8.84	08/09/2012		0.00	08/09/2012	35	6	4	11	33
Carys 2.2	4	08/08/2012	22.40	44.80	26/08/2012	25.06	50.12	06/09/2012	25.50	51.00	0.88	08/09/2012	24.88	49.76	28/08/2012	12	7	4	18	0
Carys 2.3	8	08/08/2012	16.48	32.96	26/08/2012	17.14	34.28	06/09/2012	17.46	34.92	0.64	08/09/2012	17.50	35.00	28/08/2012					
Carys 2.4	12	08/08/2012	12.08	24.16	26/08/2012	16.40	32.80	06/09/2012	19.98	39.96	7.16	08/09/2012	19.06	38.12	28/08/2012	8	6	2.5	10	19
Carys 2.5	14	08/08/2012	17.08	34.16	26/08/2012	20.38	40.76	06/09/2012	21.53	43.06	2.30	08/09/2012	21.72	43.44	02/09/2012	0	7	4	15	1
Carys 2.6	17	08/08/2012	14.40	28.80	26/08/2012	17.88	35.76	06/09/2012	19.60	39.20	3.44	08/09/2012	19.68	39.36	02/09/2012	5	6	4	9	0
Carys 2.7	376	08/08/2012	16.64	33.28	26/08/2012	16.38	32.76	06/09/2012	17.86	35.72	2.96	08/09/2012	17.50	35.00	02/09/2012	12	6	4	12	0
Carys 2.8	18	08/08/2012	16.50	33.00	26/08/2012	18.70	37.40	06/09/2012	21.40	42.80	5.40	08/09/2012	20.18	40.36	02/09/2012	3	6	3	12	2
Carys 2.9	20	08/08/2012	20.40	40.80	26/08/2012	15.28	30.56	06/09/2012	17.30	34.60	4.04	08/09/2012	18.18	36.36	02/09/2012		4	2	10	21
Carys 2.10	21	08/08/2012	16.72	33.44	26/08/2012	17.14	34.28	06/09/2012	17.34	34.68	0.40	08/09/2012	18.22	36.44	02/09/2012	25	5	2	7	44
Carys 2.11	372	08/08/2012	20.44	40.88	26/08/2012	21.90	43.80	06/09/2012	21.38	42.76	-1.04	08/09/2012	21.65	43.30	02/09/2012					13
Carys 2.12	378	08/08/2012	17.30	34.60	26/08/2012	18.08	36.16	06/09/2012	20.04	40.08	3.92	08/09/2012	20.10	40.20	02/09/2012		6	3	13	3
Carys 2.13	421	08/08/2012	18.22	36.44	26/08/2012	19.06	38.12	06/09/2012	18.49	36.98	-1.14	08/09/2012	18.54	37.08	02/09/2012					25
Carys 2.14	31	08/08/2012	16.66	33.32	26/08/2012	20.38	40.76	06/09/2012	18.28	36.56	-4.20	08/09/2012		0.00	03/09/2012		8	5	15	
Carys 2.15	38	08/08/2012	16.38	32.76	26/08/2012	18.02	36.04	06/09/2012	16.54	33.08	-2.96	08/09/2012	16.46	32.92	03/09/2012	3	6	4	8	2
Carys 2.16	336	08/08/2012	18.1	36.20	26/08/2012	18.28	36.56	06/09/2012	17.5	35.00	-1.56	08/09/2012	17.22	34.44	03/09/2012	4	7	4	13	12
Carys 2.17	39	08/08/2012	16.8	33.60	26/08/2012	17.5	35.00	06/09/2012	16.5	33.00	-2.00	08/09/2012	15.44	30.88	03/09/2012		7	4	10	6
Carys 2.18	40	08/08/2012	18.7	37.40	26/08/2012	21.3	42.60	06/09/2012	20.45	40.90	-1.70	08/09/2012	20.15	40.30	03/09/2012	12	7	4	10	28
Carys 2.19	42	08/08/2012	17.78	35.56	26/08/2012	18.16	36.32	06/09/2012	17.66	35.32	-1.00	08/09/2012	16.10	32.20	03/09/2012	7	5	3	9	38
Carys 2.20	43	08/08/2012	15.6	31.20	26/08/2012	17.65	35.30	06/09/2012	19.42	38.84	3.54	08/09/2012	19.54	39.08	03/09/2012		7	4	10	40
Carys 2.21	50			0.00	26/08/2012	22.3	44.60	06/09/2012	17.88	35.76	-8.84	08/09/2012	18.70	37.40	03/09/2012	35	6	4	11	33

Appendix ii continued. 2012 colony data

MotherID	ColonyID	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Date	Weight Measured	Hive Weight Kg	Weight change 26/8-6/9	Date	Weight Measured	Hive Weight Kg	Date	Varroa Count	Frames w bees	Frames w brood	brood	Temperament LLandegla 4/9/12
Catrin 2.1	16	08/08/2012	20.40	40.80	26/08/2012	22.54	45.08	06/09/2012	22.42	44.84	-0.24	08/09/2012	22.20	44.40	02/09/2012	3	8	5	10	0
Catrin 2.2	19	08/08/2012	13.42	26.84	26/08/2012	16.20	32.40	06/09/2012	20.04	40.08	7.68	08/09/2012	19.58	39.16	02/09/2012	4	6	3	12	0
Catrin 2.3	24	08/08/2012	20.66	41.32	26/08/2012	21.80	43.60	06/09/2012	21.56	43.12	-0.48	08/09/2012	21.78	43.56	02/09/2012		7	4	15	4
Catrin 2.4	381	08/08/2012	19.66	39.32	26/08/2012	23.06	46.12	06/09/2012	25.18	50.36	4.24	08/09/2012	23.40	46.80	02/09/2012	35	6	4	7	8
Catrin 2.5	28	08/08/2012	16.32	32.64	26/08/2012	18.26	36.52	06/09/2012	22.18	44.36	7.84	08/09/2012	16.70	33.40	02/09/2012		6	3	8	5
Catrin 2.6	261	08/08/2012	19.1	38.20	26/08/2012	20.92	41.84	06/09/2012	18.7	37.40	-4.44	08/09/2012	18.50	37.00	03/09/2012	3	8	4	16	21
Catrin 2.7	35	08/08/2012	22.16	44.32	26/08/2012	24.32	48.64	06/09/2012	24.68	49.36	0.72	08/09/2012	23.24	46.48	03/09/2012	40	7	3		4
Catrin 2.8	41	08/08/2012	17.3	34.60	26/08/2012	18.6	37.20	06/09/2012	18.3	36.60	-0.60	08/09/2012	17.90	35.80	03/09/2012	15	8	5	15	
Catrin 2.9	44	08/08/2012	16.64	33.28	26/08/2012	18.48	36.96	06/09/2012	18.04	36.08	-0.88	08/09/2012	17.80	35.60	03/09/2012	7	8.5	4	10	28
Llinos 2.1	5	08/08/2012	18.50	37.00	26/08/2012	18.42	36.84	06/09/2012	18.78	37.56	0.72	08/09/2012	19.14	38.28	28/08/2012	2	6	3	12	6
Llinos 2.2	6	08/08/2012	15.54	31.08	26/08/2012	16.80	33.60	06/09/2012	16.90	33.80	0.20	08/09/2012	16.36	32.72	28/08/2012	5	7	4		0
Llinos 2.3	7	08/08/2012	15.38	30.76	26/08/2012	17.14	34.28	06/09/2012	20.10	40.20	5.92	08/09/2012	19.02	38.04	28/08/2012	15	6	2.5		0
Llinos 2.4	10	08/08/2012	16.54	33.08	26/08/2012	21.20	42.40	06/09/2012	22.44	44.88	2.48	08/09/2012	21.36	42.72	28/08/2012		8	4	16	0
Llinos 2.5	507	08/08/2012	17.08	34.16	26/08/2012	20.20	40.40	06/09/2012	20.23	40.46	0.06	08/09/2012	20.66	41.32	02/09/2012	5	8	3	10	0
Llinos 2.6	15	08/08/2012	16.44	32.88	26/08/2012	19.32	38.64	06/09/2012	19.90	39.80	1.16	08/09/2012	19.88	39.76	02/09/2012	8	7	3	12	0
Llinos 2.7	22	08/08/2012	21.10	42.20	26/08/2012	23.90	47.80	06/09/2012	24.70	49.40	1.60	08/09/2012	25.11	50.22	02/09/2012		6	3	7	0
Llinos 2.8	25	08/08/2012	13.85	27.70	26/08/2012	15.58	31.16	06/09/2012	15.46	30.92	-0.24	08/09/2012	14.22	28.44	02/09/2012		6	2	4	11
Llinos 2.9	29	08/08/2012	17.90	35.80	26/08/2012	20.20	40.40	06/09/2012	23.50	47.00	6.60	08/09/2012		0.00	03/09/2012	5	7	3	10	4
Llinos 2.10	30	08/08/2012	18.32	36.64	26/08/2012	21.81	43.62	06/09/2012	26.20	52.40	8.78	08/09/2012		0.00	03/09/2012	9	8	5	10	0
Llinos 2.11	32	08/08/2012	15.01	30.02	26/08/2012	15.78	31.56	06/09/2012	15.18	30.36	-1.20	08/09/2012	15.32	30.64	03/09/2012	28	6	3		23
Llinos 2.12	422	08/08/2012	17.06	34.12	26/08/2012	20.32	40.64	06/09/2012	20.48	40.96	0.32	08/09/2012	20.00	40.00	03/09/2012	5	6	3	10	0
Llinos 2.13	34	08/08/2012	18.14	36.28	26/08/2012	20.54	41.08	06/09/2012	20.18	40.36	-0.72	08/09/2012	20.64	41.28	03/09/2012	16	6	4		2
Llinos 2.14	36	08/08/2012	17.38	34.76	26/08/2012	21.2	42.40	06/09/2012	23.14	46.28	3.88	08/09/2012	22.40	44.80	03/09/2012		7	4	14	1
Llinos 2.15	432	08/08/2012	18.7	37.40	26/08/2012	21.29	42.58	06/09/2012	20.58	41.16	-1.42	08/09/2012	21.18	42.36	03/09/2012	22	8	4	12	11
Llinos 2.17	45	08/08/2012	18.5	37.00	26/08/2012	22.65	45.30	06/09/2012	22.36	44.72	-0.58	08/09/2012	21.10	42.20	03/09/2012		7	4	11	6
Llinos 2.18	46	08/08/2012	19.38	38.76	26/08/2012	19.84	39.68	06/09/2012	21.34	42.68	3.00	08/09/2012	20.48	40.96	03/09/2012		6.5	2	4	14

Appendix III; Matlab code for Microsatellite simulation model

```
%Microsat model input allele frequencies
nqueens = 100;
queenalleles=2;
nobreeders1=8;
nobreeders2=4;
nobreeders3=4;
nobreeders4=4;
nobreeders5=4;
nodrones=7;
a = 1000; %number of iterations
for freqallele1=(1:a);
     freqallele2=(1:a);
         freqallele3=(1:a);
             freqallele4=(1:a);
                freqallele5=(1:a);
                    freqallele6=(1:a);
                        freqallele7=(1:a);
                            freqallele8=(1:a);
                                 freqallele9=(1:a);
                                     freqallele10=(1:a);
                                        freqallele11=(1:a);
                                           freqallele12=(1:a);

% ENTER ALLELE FREQUENCY DISTRIBUTION HERE

Distributiond=rand(nqueens,nodrones);
allele1d_0=(Distributiond>0) & (Distributiond<0.038);
allele2d_0=(Distributiond>=0.038) & (Distributiond<0.113);
allele3d_0=(Distributiond>=0.113) & (Distributiond<0.236);
allele4d_0=(Distributiond>=0.236) & (Distributiond<0.311);
allele5d_0=(Distributiond>=0.311) & (Distributiond<0.425);
allele6d_0=(Distributiond>=0.425) & (Distributiond<0.491);
allele7d_0=(Distributiond>=0.491) & (Distributiond<0.774);
allele8d_0=(Distributiond>=0.774) & (Distributiond<0.896);
allele9d_0=(Distributiond>=0.896) & (Distributiond<0.962);
allele10d_0=(Distributiond>=0.962) & (Distributiond<0.991);
allele11d_0=(Distributiond>=0.991) & (Distributiond<1);
allele12d_0=(Distributiond>=1) & (Distributiond<1);

count_allele1d=sum(allele1d_0);
count_allele2d=sum(allele2d_0);
count_allele3d=sum(allele3d_0);
count_allele4d=sum(allele4d_0);
count_allele5d=sum(allele5d_0);
count_allele6d=sum(allele6d_0);
count_allele7d=sum(allele7d_0);
count_allele8d=sum(allele8d_0);
count_allele9d=sum(allele9d_0);
count_allele10d=sum(allele10d_0);
count_allele11d=sum(allele11d_0);
count_allele12d=sum(allele12d_0);
```

```
freqallele1d_0=sum(count_allele1d)/(nqueens*nodrones);
freqallele2d_0=sum(count_allele2d)/(nqueens*nodrones);
freqallele3d_0=sum(count_allele3d)/(nqueens*nodrones);
freqallele4d_0=sum(count_allele4d)/(nqueens*nodrones);
freqallele5d_0=sum(count_allele5d)/(nqueens*nodrones);
freqallele6d_0=sum(count_allele6d)/(nqueens*nodrones);
freqallele7d_0=sum(count_allele7d)/(nqueens*nodrones);
freqallele8d_0=sum(count_allele8d)/(nqueens*nodrones);
freqallele9d_0=sum(count_allele9d)/(nqueens*nodrones);
freqallele10d_0=sum(count_allele10d)/(nqueens*nodrones);
freqallele11d_0=sum(count_allele11d)/(nqueens*nodrones);
freqallele12d_0=sum(count_allele12d)/(nqueens*nodrones);


%Describe baseline queen allele frequency dist

Distribution=rand(nqueens,queenalleles);

allele1q_0=(Distribution>0) & (Distribution<0.038);
allele2q_0=(Distribution>=0.038) & (Distribution<0.113);
allele3q_0=(Distribution>=0.113) & (Distribution<0.236);
allele4q_0=(Distribution>=0.236) & (Distribution<0.311);
allele5q_0=(Distribution>=0.311) & (Distribution<0.425);
allele6q_0=(Distribution>=0.425) & (Distribution<0.491);
allele7q_0=(Distribution>=0.491) & (Distribution<0.774);
allele8q_0=(Distribution>=0.774) & (Distribution<0.896);
allele9q_0=(Distribution>=0.896) & (Distribution<0.962);
allele10q_0=(Distribution>=0.962) & (Distribution<0.991);
allele11q_0=(Distribution>=0.991) & (Distribution<1);
allele12q_0=(Distribution>=1) & (Distribution<1);

count_allele1q_0=sum(allele1q_0);
count_allele2q_0=sum(allele2q_0);
count_allele3q_0=sum(allele3q_0);
count_allele4q_0=sum(allele4q_0);
count_allele5q_0=sum(allele5q_0);
count_allele6q_0=sum(allele6q_0);
count_allele7q_0=sum(allele7q_0);
count_allele8q_0=sum(allele8q_0);
count_allele9q_0=sum(allele9q_0);
count_allele10q_0=sum(allele10q_0);
count_allele11q_0=sum(allele11q_0);
count_allele12q_0=sum(allele12q_0);

freqallele1q_0=sum(count_allele1q_0)/(nqueens*queenalleles);
freqallele2q_0=sum(count_allele2q_0)/(nqueens*queenalleles);
freqallele3q_0=sum(count_allele3q_0)/(nqueens*queenalleles);
freqallele4q_0=sum(count_allele4q_0)/(nqueens*queenalleles);
freqallele5q_0=sum(count_allele5q_0)/(nqueens*queenalleles);
freqallele6q_0=sum(count_allele6q_0)/(nqueens*queenalleles);
freqallele7q_0=sum(count_allele7q_0)/(nqueens*queenalleles);
freqallele8q_0=sum(count_allele8q_0)/(nqueens*queenalleles);
freqallele9q_0=sum(count_allele9q_0)/(nqueens*queenalleles);
```

```
freqallele10q_0=sum(count_allele10q_0)/(nqueens*queenalleles);
freqallele11q_0=sum(count_allele11q_0)/(nqueens*queenalleles);
freqallele12q_0=sum(count_allele12q_0)/(nqueens*queenalleles);

%generate overall simulated population level gene distribution by adding
%the weighted values of drone and queen derived alleles

freqallele1_0=((2*freqallele1d_0+freqallele1q_0)/3)
freqallele2_0=((2*freqallele2d_0+freqallele2q_0)/3)
freqallele3_0=((2*freqallele3d_0+freqallele3q_0)/3)
freqallele4_0=((2*freqallele4d_0+freqallele4q_0)/3)
freqallele5_0=((2*freqallele5d_0+freqallele5q_0)/3)
freqallele6_0=((2*freqallele6d_0+freqallele6q_0)/3)
freqallele7_0=((2*freqallele7d_0+freqallele7q_0)/3)
freqallele8_0=((2*freqallele8d_0+freqallele8q_0)/3)
freqallele9_0=((2*freqallele9d_0+freqallele9q_0)/3)
freqallele10_0=((2*freqallele10d_0+freqallele10q_0)/3)
freqallele11_0=((2*freqallele11d_0+freqallele11q_0)/3)
freqallele12_0=((2*freqallele12d_0+freqallele12q_0)/3)



mat1_G0(freqallele1)=freqallele1_0
mat2_G0(freqallele1)=freqallele2_0
mat3_G0(freqallele1)=freqallele3_0
mat4_G0(freqallele1)=freqallele4_0
mat5_G0(freqallele1)=freqallele5_0
mat6_G0(freqallele1)=freqallele6_0
mat7_G0(freqallele1)=freqallele7_0
mat8_G0(freqallele1)=freqallele8_0
mat9_G0(freqallele1)=freqallele9_0
mat10_G0(freqallele1)=freqallele10_0
mat11_G0(freqallele1)=freqallele11_0
mat12_G0(freqallele1)=freqallele12_0

Breeders1=datasample(Distribution,nobreeders1);

allele1q_1=(Breeders1>0) & (Breeders1<freqallele1_0);
allele2q_1=(Breeders1>=freqallele1_0) &
(Breeders1<(freqallele1_0+freqallele2_0));
allele3q_1=(Breeders1>=(freqallele1_0+freqallele2_0)) &
(Breeders1<(freqallele1_0+freqallele2_0+freqallele3_0));
allele4q_1=(Breeders1>=(freqallele1_0+freqallele2_0+freqallele3_0)) &
(Breeders1<(freqallele1_0+freqallele2_0+freqallele3_0+freqallele4_0));
allele5q_1=(Breeders1>=(freqallele1_0+freqallele2_0+freqallele3_0+freqalle
le4_0)) &
(Breeders1<(freqallele1_0+freqallele2_0+freqallele3_0+freqallele4_0+freqal
lele5_0));
allele6q_1=(Breeders1>=(freqallele1_0+freqallele2_0+freqallele3_0+freqalle
le4_0+freqallele5_0)) &
(Breeders1<(freqallele1_0+freqallele2_0+freqallele3_0+freqallele4_0+freqal
lele5_0+freqallele6_0));
allele7q_1=(Breeders1>=(freqallele1_0+freqallele2_0+freqallele3_0+freqalle
le4_0+freqallele5_0+freqallele6_0)) &
```

```
(Breeders1<(freqallele1_0+freqallele2_0+freqallele3_0+freqallele4_0+freqal
lele5_0+freqallele6_0+freqallele7_0));
allele8q_1=(Breeders1>=(freqallele1_0+freqallele2_0+freqallele3_0+freqalle
le4_0+freqallele5_0+freqallele6_0+freqallele7_0)) &
(Breeders1<(freqallele1_0+freqallele2_0+freqallele3_0+freqallele4_0+freqal
lele5_0+freqallele6_0+freqallele7_0+freqallele8_0));
allele9q_1=(Breeders1>=(freqallele1_0+freqallele2_0+freqallele3_0+freqalle
le4_0+freqallele5_0+freqallele6_0+freqallele7_0+freqallele8_0)) &
(Breeders1<(freqallele1_0+freqallele2_0+freqallele3_0+freqallele4_0+freqal
lele5_0+freqallele6_0+freqallele7_0+freqallele8_0+freqallele9_0));
allele10q_1=(Breeders1>=(freqallele1_0+freqallele2_0+freqallele3_0+freqall
ele4_0+freqallele5_0+freqallele6_0+freqallele7_0+freqallele8_0+freqallele9
_0)) &
(Breeders1<(freqallele1_0+freqallele2_0+freqallele3_0+freqallele4_0+freqal
lele5_0+freqallele6_0+freqallele7_0+freqallele8_0+freqallele9_0+freqallele
10_0));
allele11q_1=(Breeders1>=(freqallele1_0+freqallele2_0+freqallele3_0+freqall
ele4_0+freqallele5_0+freqallele6_0+freqallele7_0+freqallele8_0+freqallele9
_0+freqallele10_0)) &
(Breeders1<(freqallele1_0+freqallele2_0+freqallele3_0+freqallele4_0+freqal
lele5_0+freqallele6_0+freqallele7_0+freqallele8_0+freqallele9_0+freqallele
10_0+freqallele11_0));
allele12q_1=(Breeders1>=(freqallele1_0+freqallele2_0+freqallele3_0+freqall
ele4_0+freqallele5_0+freqallele6_0+freqallele7_0+freqallele8_0+freqallele9
_0+freqallele10_0+freqallele11_0)) & (Breeders1<1.0);

count_allele1q_1=sum(allele1q_1);
count_allele2q_1=sum(allele2q_1);
count_allele3q_1=sum(allele3q_1);
count_allele4q_1=sum(allele4q_1);
count_allele5q_1=sum(allele5q_1);
count_allele6q_1=sum(allele6q_1);
count_allele7q_1=sum(allele7q_1);
count_allele8q_1=sum(allele8q_1);
count_allele9q_1=sum(allele9q_1);
count_allele10q_1=sum(allele10q_1);
count_allele11q_1=sum(allele11q_1);
count_allele12q_1=sum(allele12q_1);


freqallele1q_1=sum(count_allele1q_1)/(nobreeders1*queenalleles);
freqallele2q_1=sum(count_allele2q_1)/(nobreeders1*queenalleles);
freqallele3q_1=sum(count_allele3q_1)/(nobreeders1*queenalleles);
freqallele4q_1=sum(count_allele4q_1)/(nobreeders1*queenalleles);
freqallele5q_1=sum(count_allele5q_1)/(nobreeders1*queenalleles);
freqallele6q_1=sum(count_allele6q_1)/(nobreeders1*queenalleles);
freqallele7q_1=sum(count_allele7q_1)/(nobreeders1*queenalleles);
freqallele8q_1=sum(count_allele8q_1)/(nobreeders1*queenalleles);
freqallele9q_1=sum(count_allele9q_1)/(nobreeders1*queenalleles);
freqallele10q_1=sum(count_allele10q_1)/(nobreeders1*queenalleles);
freqallele11q_1=sum(count_allele11q_1)/(nobreeders1*queenalleles);
freqallele12q_1=sum(count_allele12q_1)/(nobreeders1*queenalleles);

Ballelefreq1_G1=(2*freqallele1q_1+freqallele1d_0)/3
```

```
Ballelefreq2_G1=(2*freqallele2q_1+freqallele2d_0)/3
Ballelefreq3_G1=(2*freqallele3q_1+freqallele3d_0)/3
Ballelefreq4_G1=(2*freqallele4q_1+freqallele4d_0)/3
Ballelefreq5_G1=(2*freqallele5q_1+freqallele5d_0)/3
Ballelefreq6_G1=(2*freqallele6q_1+freqallele6d_0)/3
Ballelefreq7_G1=(2*freqallele7q_1+freqallele7d_0)/3
Ballelefreq8_G1=(2*freqallele8q_1+freqallele8d_0)/3
Ballelefreq9_G1=(2*freqallele9q_1+freqallele9d_0)/3
Ballelefreq10_G1=(2*freqallele10q_1+freqallele10d_0)/3
Ballelefreq11_G1=(2*freqallele11q_1+freqallele11d_0)/3
Ballelefreq12_G1=(2*freqallele12q_1+freqallele12d_0)/3



%GENERATE 100 QUEENS WITH ABOVE DISTRIBUTION
DistGen1Q=rand(nqueens,queenalleles) ;

allele1q_1=(DistGen1Q>0) & (DistGen1Q<Ballelefreq1_G1);
allele2q_1=(DistGen1Q>=Ballelefreq1_G1) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1));
allele3q_1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1));
allele4q_1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1))
&
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1));
allele5q_1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ba
llelefreq4_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1));
allele6q_1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ba
llelefreq4_G1+Ballelefreq5_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1));
allele7q_1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ba
llelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1));
allele8q_1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ba
llelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1));
allele9q_1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ba
llelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8
_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballelef
req9_G1));
allele10q_1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+B
allelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq
8_G1+Ballelefreq9_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballelef
req9_G1+Ballelefreq10_G1));
```

```
allele11q_1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+B
allelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq
8_G1+Ballelefreq9_G1+Ballelefreq10_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballelef
req9_G1+Ballelefreq10_G1+Ballelefreq11_G1));
allele12q_1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+B
allelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq
8_G1+Ballelefreq9_G1+Ballelefreq10_G1+Ballelefreq11_G1)) & (DistGen1Q<1);

count_allele1q_1=sum(allele1q_1);
count_allele2q_1=sum(allele2q_1);
count_allele3q_1=sum(allele3q_1);
count_allele4q_1=sum(allele4q_1);
count_allele5q_1=sum(allele5q_1);
count_allele6q_1=sum(allele6q_1);
count_allele7q_1=sum(allele7q_1);
count_allele8q_1=sum(allele8q_1);
count_allele9q_1=sum(allele9q_1);
count_allele10q_1=sum(allele10q_1);
count_allele11q_1=sum(allele11q_1);
count_allele12q_1=sum(allele12q_1);

freqallele1q_1=sum(count_allele1q_1)/(nqueens*queenalleles)
freqallele2q_1=sum(count_allele2q_1)/(nqueens*queenalleles)
freqallele3q_1=sum(count_allele3q_1)/(nqueens*queenalleles)
freqallele4q_1=sum(count_allele4q_1)/(nqueens*queenalleles)
freqallele5q_1=sum(count_allele5q_1)/(nqueens*queenalleles)
freqallele6q_1=sum(count_allele6q_1)/(nqueens*queenalleles)
freqallele7q_1=sum(count_allele7q_1)/(nqueens*queenalleles)
freqallele8q_1=sum(count_allele8q_1)/(nqueens*queenalleles)
freqallele9q_1=sum(count_allele9q_1)/(nqueens*queenalleles)
freqallele10q_1=sum(count_allele10q_1)/(nqueens*queenalleles)
freqallele11q_1=sum(count_allele11q_1)/(nqueens*queenalleles)
freqallele12q_1=sum(count_allele12q_1)/(nqueens*queenalleles)

freqallele1_1=((2*freqallele1q_1+freqallele1d_0)/3)
freqallele2_1=((2*freqallele2q_1+freqallele2d_0)/3)
freqallele3_1=((2*freqallele3q_1+freqallele3d_0)/3)
freqallele4_1=((2*freqallele4q_1+freqallele4d_0)/3)
freqallele5_1=((2*freqallele5q_1+freqallele5d_0)/3)
freqallele6_1=((2*freqallele6q_1+freqallele6d_0)/3)
freqallele7_1=((2*freqallele7q_1+freqallele7d_0)/3)
freqallele8_1=((2*freqallele8q_1+freqallele8d_0)/3)
freqallele9_1=((2*freqallele9q_1+freqallele9d_0)/3)
freqallele10_1=((2*freqallele10q_1+freqallele10d_0)/3)
freqallele11_1=((2*freqallele11q_1+freqallele11d_0)/3)
freqallele12_1=((2*freqallele12q_1+freqallele12d_0)/3)

mat1_G1(freqallele1)=freqallele1_1
mat2_G1(freqallele1)=freqallele2_1
mat3_G1(freqallele1)=freqallele3_1
mat4_G1(freqallele1)=freqallele4_1
mat5_G1(freqallele1)=freqallele5_1
```

```
mat6_G1(freqallele1)=freqallele6_1
mat7_G1(freqallele1)=freqallele7_1
mat8_G1(freqallele1)=freqallele8_1
mat9_G1(freqallele1)=freqallele9_1
mat10_G1(freqallele1)=freqallele10_1
mat11_G1(freqallele1)=freqallele11_1
mat12_G1(freqallele1)=freqallele12_1


Breeders2=datasample(Distribution,nobreeders2);

allele1q_2=(Breeders2>0) & (Breeders2<freqallele1_1);
allele2q_2=(Breeders2>=freqallele1_1) &
(Breeders2<(freqallele1_1+freqallele2_1));
allele3q_2=(Breeders2>=(freqallele1_1+freqallele2_1)) &
(Breeders2<(freqallele1_1+freqallele2_1+freqallele3_1));
allele4q_2=(Breeders2>=(freqallele1_1+freqallele2_1+freqallele3_1)) &
(Breeders2<(freqallele1_1+freqallele2_1+freqallele3_1+freqallele4_1));
allele5q_2=(Breeders2>=(freqallele1_1+freqallele2_1+freqallele3_1+freqalle
le4_1)) &
(Breeders2<(freqallele1_1+freqallele2_1+freqallele3_1+freqallele4_1+freqal
lele5_1));
allele6q_2=(Breeders2>=(freqallele1_1+freqallele2_1+freqallele3_1+freqalle
le4_1+freqallele5_1)) &
(Breeders2<(freqallele1_1+freqallele2_1+freqallele3_1+freqallele4_1+freqal
lele5_1+freqallele6_1));
allele7q_2=(Breeders2>=(freqallele1_1+freqallele2_1+freqallele3_1+freqalle
le4_1+freqallele5_1+freqallele6_1)) &
(Breeders2<(freqallele1_1+freqallele2_1+freqallele3_1+freqallele4_1+freqal
lele5_1+freqallele6_1+freqallele7_1));
allele8q_2=(Breeders2>=(freqallele1_1+freqallele2_1+freqallele3_1+freqalle
le4_1+freqallele5_1+freqallele6_1+freqallele7_1)) &
(Breeders2<(freqallele1_1+freqallele2_1+freqallele3_1+freqallele4_1+freqal
lele5_1+freqallele6_1+freqallele7_1+freqallele8_1));
allele9q_2=(Breeders2>=(freqallele1_1+freqallele2_1+freqallele3_1+freqalle
le4_1+freqallele5_1+freqallele6_1+freqallele7_1+freqallele8_1)) &
(Breeders2<(freqallele1_1+freqallele2_1+freqallele3_1+freqallele4_1+freqal
lele5_1+freqallele6_1+freqallele7_1+freqallele8_1+freqallele9_1));
allele10q_2=(Breeders2>=(freqallele1_1+freqallele2_1+freqallele3_1+freqall
ele4_1+freqallele5_1+freqallele6_1+freqallele7_1+freqallele8_1+freqallele9
_1)) &
(Breeders2<(freqallele1_1+freqallele2_1+freqallele3_1+freqallele4_1+freqal
lele5_1+freqallele6_1+freqallele7_1+freqallele8_1+freqallele9_1+freqallele
10_1));
allele11q_2=(Breeders2>=(freqallele1_1+freqallele2_1+freqallele3_1+freqall
ele4_1+freqallele5_1+freqallele6_1+freqallele7_1+freqallele8_1+freqallele9
_1+freqallele10_1)) &
(Breeders2<(freqallele1_1+freqallele2_1+freqallele3_1+freqallele4_1+freqal
lele5_1+freqallele6_1+freqallele7_1+freqallele8_1+freqallele9_1+freqallele
10_1+freqallele11_1));
allele12q_2=(Breeders2>=(freqallele1_1+freqallele2_1+freqallele3_1+freqall
ele4_1+freqallele5_1+freqallele6_1+freqallele7_1+freqallele8_1+freqallele9
_1+freqallele10_1+freqallele11_1)) & (Breeders2<1.0);
```

```
count_allele1q_2=sum(allele1q_2);
count_allele2q_2=sum(allele2q_2);
count_allele3q_2=sum(allele3q_2);
count_allele4q_2=sum(allele4q_2);
count_allele5q_2=sum(allele5q_2);
count_allele6q_2=sum(allele6q_2);
count_allele7q_2=sum(allele7q_2);
count_allele8q_2=sum(allele8q_2);
count_allele9q_2=sum(allele9q_2);
count_allele10q_2=sum(allele10q_2);
count_allele11q_2=sum(allele11q_2);
count_allele12q_2=sum(allele12q_2);

freqallele1q_2=sum(count_allele1q_2)/(nobreeders2*queenalleles);
freqallele2q_2=sum(count_allele2q_2)/(nobreeders2*queenalleles);
freqallele3q_2=sum(count_allele3q_2)/(nobreeders2*queenalleles);
freqallele4q_2=sum(count_allele4q_2)/(nobreeders2*queenalleles);
freqallele5q_2=sum(count_allele5q_2)/(nobreeders2*queenalleles);
freqallele6q_2=sum(count_allele6q_2)/(nobreeders2*queenalleles);
freqallele7q_2=sum(count_allele7q_2)/(nobreeders2*queenalleles);
freqallele8q_2=sum(count_allele8q_2)/(nobreeders2*queenalleles);
freqallele9q_2=sum(count_allele9q_2)/(nobreeders2*queenalleles);
freqallele10q_2=sum(count_allele10q_2)/(nobreeders2*queenalleles);
freqallele11q_2=sum(count_allele11q_2)/(nobreeders2*queenalleles);
freqallele12q_2=sum(count_allele12q_2)/(nobreeders2*queenalleles);

Ballelefreq1_G2=(2*freqallele1q_2+freqallele1q_1)/3
Ballelefreq2_G2=(2*freqallele2q_2+freqallele2q_1)/3
Ballelefreq3_G2=(2*freqallele3q_2+freqallele3q_1)/3
Ballelefreq4_G2=(2*freqallele4q_2+freqallele4q_1)/3
Ballelefreq5_G2=(2*freqallele5q_2+freqallele5q_1)/3
Ballelefreq6_G2=(2*freqallele6q_2+freqallele6q_1)/3
Ballelefreq7_G2=(2*freqallele7q_2+freqallele7q_1)/3
Ballelefreq8_G2=(2*freqallele8q_2+freqallele8q_1)/3
Ballelefreq9_G2=(2*freqallele9q_2+freqallele9q_1)/3
Ballelefreq10_G2=(2*freqallele10q_2+freqallele10q_1)/3
Ballelefreq11_G2=(2*freqallele11q_2+freqallele11q_1)/3
Ballelefreq12_G2=(2*freqallele12q_2+freqallele12q_1)/3

%GENERATE 100 NEW QUEEN GENOTYPES FROM THIS FREQUENCY DISTRIBUTION

DistGen2Q=rand(nqueens,queenalleles);

allele1q_2=(DistGen2Q>0) & (DistGen2Q<Ballelefreq1_G2);
allele2q_2=(DistGen2Q>=Ballelefreq1_G2) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2));
allele3q_2=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2));
allele4q_2=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2))
&
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2));
allele5q_2=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ba
llelefreq4_G2)) &
```

```
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2));
allele6q_2=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ba
llelefreq4_G2+Ballelefreq5_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2));
allele7q_2=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ba
llelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2));
allele8q_2=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ba
llelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2));
allele9q_2=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ba
llelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8
_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballelef
req9_G2));
allele10q_2=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+B
allelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq
8_G2+Ballelefreq9_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballelef
req9_G2+Ballelefreq10_G2));
allele11q_2=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+B
allelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq
8_G2+Ballelefreq9_G2+Ballelefreq10_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballelef
req9_G2+Ballelefreq10_G2+Ballelefreq11_G2));
allele12q_2=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+B
allelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq
8_G2+Ballelefreq9_G2+Ballelefreq10_G2+Ballelefreq11_G2)) & (DistGen2Q<1);

count_allele1q_2=sum(allele1q_2);
count_allele2q_2=sum(allele2q_2);
count_allele3q_2=sum(allele3q_2);
count_allele4q_2=sum(allele4q_2);
count_allele5q_2=sum(allele5q_2);
count_allele6q_2=sum(allele6q_2);
count_allele7q_2=sum(allele7q_2);
count_allele8q_2=sum(allele8q_2);
count_allele9q_2=sum(allele9q_2);
count_allele10q_2=sum(allele10q_2);
count_allele11q_2=sum(allele11q_2);
count_allele12q_2=sum(allele12q_2);

freqallele1q_2=sum(count_allele1q_2)/(nqueens*queenalleles);
freqallele2q_2=sum(count_allele2q_2)/(nqueens*queenalleles);
freqallele3q_2=sum(count_allele3q_2)/(nqueens*queenalleles);
freqallele4q_2=sum(count_allele4q_2)/(nqueens*queenalleles);
freqallele5q_2=sum(count_allele5q_2)/(nqueens*queenalleles);
freqallele6q_2=sum(count_allele6q_2)/(nqueens*queenalleles);
```

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freqallele7q_2=sum(count_allele7q_2)/(nqueens*queenalleles);
freqallele8q_2=sum(count_allele8q_2)/(nqueens*queenalleles);
freqallele9q_2=sum(count_allele9q_2)/(nqueens*queenalleles);
freqallele10q_2=sum(count_allele10q_2)/(nqueens*queenalleles);
freqallele11q_2=sum(count_allele11q_2)/(nqueens*queenalleles);
freqallele12q_2=sum(count_allele12q_2)/(nqueens*queenalleles);


freqallele1_2=((2*freqallele1q_2+freqallele1q_1)/3)
freqallele2_2=((2*freqallele2q_2+freqallele2q_1)/3)
freqallele3_2=((2*freqallele3q_2+freqallele3q_1)/3)
freqallele4_2=((2*freqallele4q_2+freqallele4q_1)/3)
freqallele5_2=((2*freqallele5q_2+freqallele5q_1)/3)
freqallele6_2=((2*freqallele6q_2+freqallele6q_1)/3)
freqallele7_2=((2*freqallele7q_2+freqallele7q_1)/3)
freqallele8_2=((2*freqallele8q_2+freqallele8q_1)/3)
freqallele9_2=((2*freqallele9q_2+freqallele9q_1)/3)
freqallele10_2=((2*freqallele10q_2+freqallele10q_1)/3)
freqallele11_2=((2*freqallele11q_2+freqallele11q_1)/3)
freqallele12_2=((2*freqallele12q_2+freqallele12q_1)/3)

mat1_G2(freqallele1)=freqallele1_2
mat2_G2(freqallele1)=freqallele2_2
mat3_G2(freqallele1)=freqallele3_2
mat4_G2(freqallele1)=freqallele4_2
mat5_G2(freqallele1)=freqallele5_2
mat6_G2(freqallele1)=freqallele6_2
mat7_G2(freqallele1)=freqallele7_2
mat8_G2(freqallele1)=freqallele8_2
mat9_G2(freqallele1)=freqallele9_2
mat10_G2(freqallele1)=freqallele10_2
mat11_G2(freqallele1)=freqallele11_2
mat12_G2(freqallele1)=freqallele12_2

Breeders3=datasample(Distribution,nobreeders3);
allele1q_3=(Breeders3>0) & (Breeders3<freqallele1_2);
allele2q_3=(Breeders3>=freqallele1_2) &
(Breeders3<(freqallele1_2+freqallele2_2));
allele3q_3=(Breeders3>=(freqallele1_2+freqallele2_2)) &
(Breeders3<(freqallele1_2+freqallele2_2+freqallele3_2));
allele4q_3=(Breeders3>=(freqallele1_2+freqallele2_2+freqallele3_2)) &
(Breeders3<(freqallele1_2+freqallele2_2+freqallele3_2+freqallele4_2));
allele5q_3=(Breeders3>=(freqallele1_2+freqallele2_2+freqallele3_2+freqalle
le4_2)) &
(Breeders3<(freqallele1_2+freqallele2_2+freqallele3_2+freqallele4_2+freqal
lele5_2));
allele6q_3=(Breeders3>=(freqallele1_2+freqallele2_2+freqallele3_2+freqalle
le4_2+freqallele5_2)) &
(Breeders3<(freqallele1_2+freqallele2_2+freqallele3_2+freqallele4_2+freqal
lele5_2+freqallele6_2));
allele7q_3=(Breeders3>=(freqallele1_2+freqallele2_2+freqallele3_2+freqalle
le4_2+freqallele5_2+freqallele6_2)) &
(Breeders3<(freqallele1_2+freqallele2_2+freqallele3_2+freqallele4_2+freqal
lele5_2+freqallele6_2+freqallele7_2));
```

```
allele8q_3=(Breeders3>=(freqallele1_2+freqallele2_2+freqallele3_2+freqalle
le4_2+freqallele5_2+freqallele6_2+freqallele7_2)) &
(Breeders3<(freqallele1_2+freqallele2_2+freqallele3_2+freqallele4_2+freqal
lele5_2+freqallele6_2+freqallele7_2+freqallele8_2));
allele9q_3=(Breeders3>=(freqallele1_2+freqallele2_2+freqallele3_2+freqalle
le4_2+freqallele5_2+freqallele6_2+freqallele7_2+freqallele8_2)) &
(Breeders3<(freqallele1_2+freqallele2_2+freqallele3_2+freqallele4_2+freqal
lele5_2+freqallele6_2+freqallele7_2+freqallele8_2+freqallele9_2));
allele10q_3=(Breeders3>=(freqallele1_2+freqallele2_2+freqallele3_2+freqall
ele4_2+freqallele5_2+freqallele6_2+freqallele7_2+freqallele8_2+freqallele9
_2)) &
(Breeders3<(freqallele1_2+freqallele2_2+freqallele3_2+freqallele4_2+freqal
lele5_2+freqallele6_2+freqallele7_2+freqallele8_2+freqallele9_2+freqallele
10_2));
allele11q_3=(Breeders3>=(freqallele1_2+freqallele2_2+freqallele3_2+freqall
ele4_2+freqallele5_2+freqallele6_2+freqallele7_2+freqallele8_2+freqallele9
_2+freqallele10_2)) &
(Breeders3<(freqallele1_2+freqallele2_2+freqallele3_2+freqallele4_2+freqal
lele5_2+freqallele6_2+freqallele7_2+freqallele8_2+freqallele9_2+freqallele
10_2+freqallele11_2));
allele12q_3=(Breeders3>=(freqallele1_2+freqallele2_2+freqallele3_2+freqall
ele4_2+freqallele5_2+freqallele6_2+freqallele7_2+freqallele8_2+freqallele9
_2+freqallele10_2+freqallele11_2)) & (Breeders3<1.0);


count_allele1q_3=sum(allele1q_3);
count_allele2q_3=sum(allele2q_3);
count_allele3q_3=sum(allele3q_3);
count_allele4q_3=sum(allele4q_3);
count_allele5q_3=sum(allele5q_3);
count_allele6q_3=sum(allele6q_3);
count_allele7q_3=sum(allele7q_3);
count_allele8q_3=sum(allele8q_3);
count_allele9q_3=sum(allele9q_3);
count_allele10q_3=sum(allele10q_3);
count_allele11q_3=sum(allele11q_3);
count_allele12q_3=sum(allele12q_3);

freqallele1q_3=sum(count_allele1q_3)/(nobreeders3*queenalleles);
freqallele2q_3=sum(count_allele2q_3)/(nobreeders3*queenalleles);
freqallele3q_3=sum(count_allele3q_3)/(nobreeders3*queenalleles);
freqallele4q_3=sum(count_allele4q_3)/(nobreeders3*queenalleles);
freqallele5q_3=sum(count_allele5q_3)/(nobreeders3*queenalleles);
freqallele6q_3=sum(count_allele6q_3)/(nobreeders3*queenalleles);
freqallele7q_3=sum(count_allele7q_3)/(nobreeders3*queenalleles);
freqallele8q_3=sum(count_allele8q_3)/(nobreeders3*queenalleles);
freqallele9q_3=sum(count_allele9q_3)/(nobreeders3*queenalleles);
freqallele10q_3=sum(count_allele10q_3)/(nobreeders3*queenalleles);
freqallele11q_3=sum(count_allele11q_3)/(nobreeders3*queenalleles);
freqallele12q_3=sum(count_allele12q_3)/(nobreeders3*queenalleles);

Ballelefreq1_G3=(2*freqallele1q_3+freqallele1q_2)/3
Ballelefreq2_G3=(2*freqallele2q_3+freqallele2q_2)/3
Ballelefreq3_G3=(2*freqallele3q_3+freqallele3q_2)/3
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Ballelefreq4_G3=(2*freqallele4q_3+freqallele4q_2)/3
Ballelefreq5_G3=(2*freqallele5q_3+freqallele5q_2)/3
Ballelefreq6_G3=(2*freqallele6q_3+freqallele6q_2)/3
Ballelefreq7_G3=(2*freqallele7q_3+freqallele7q_2)/3
Ballelefreq8_G3=(2*freqallele8q_3+freqallele8q_2)/3
Ballelefreq9_G3=(2*freqallele9q_3+freqallele9q_2)/3
Ballelefreq10_G3=(2*freqallele10q_3+freqallele10q_2)/3
Ballelefreq11_G3=(2*freqallele11q_3+freqallele11q_2)/3
Ballelefreq12_G3=(2*freqallele12q_3+freqallele12q_2)/3

%GENERATE 100 QUEENS
DistGen3Q=rand(nqueens,queenalleles);

allele1q_3=(DistGen3Q>0) & (DistGen3Q<Ballelefreq1_G3);
allele2q_3=(DistGen3Q>=Ballelefreq1_G3) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3));
allele3q_3=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3));
allele4q_3=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3))
&
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3));
allele5q_3=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ba
llelefreq4_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3));
allele6q_3=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ba
llelefreq4_G3+Ballelefreq5_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3+Ballelefreq6_G3));
allele7q_3=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ba
llelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3));
allele8q_3=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ba
llelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3));
allele9q_3=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ba
llelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8
_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballelef
req9_G3));
allele10q_3=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+B
allelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq
8_G3+Ballelefreq9_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballelef
req9_G3+Ballelefreq10_G3));
allele11q_3=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+B
allelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq
8_G3+Ballelefreq9_G3+Ballelefreq10_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
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3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballelef
req9_G3+Ballelefreq10_G3+Ballelefreq11_G3));
allele12q_3=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+B
allelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq
8_G3+Ballelefreq9_G3+Ballelefreq10_G3+Ballelefreq11_G3)) & (DistGen3Q<1);

count_allele1q_3=sum(allele1q_3);
count_allele2q_3=sum(allele2q_3);
count_allele3q_3=sum(allele3q_3);
count_allele4q_3=sum(allele4q_3);
count_allele5q_3=sum(allele5q_3);
count_allele6q_3=sum(allele6q_3);
count_allele7q_3=sum(allele7q_3);
count_allele8q_3=sum(allele8q_3);
count_allele9q_3=sum(allele9q_3);
count_allele10q_3=sum(allele10q_3);
count_allele11q_3=sum(allele11q_3);
count_allele12q_3=sum(allele12q_3);

freqallele1q_3=sum(count_allele1q_3)/(nqueens*queenalleles);
freqallele2q_3=sum(count_allele2q_3)/(nqueens*queenalleles);
freqallele3q_3=sum(count_allele3q_3)/(nqueens*queenalleles);
freqallele4q_3=sum(count_allele4q_3)/(nqueens*queenalleles);
freqallele5q_3=sum(count_allele5q_3)/(nqueens*queenalleles);
freqallele6q_3=sum(count_allele6q_3)/(nqueens*queenalleles);
freqallele7q_3=sum(count_allele7q_3)/(nqueens*queenalleles);
freqallele8q_3=sum(count_allele8q_3)/(nqueens*queenalleles);
freqallele9q_3=sum(count_allele9q_3)/(nqueens*queenalleles);
freqallele10q_3=sum(count_allele10q_3)/(nqueens*queenalleles);
freqallele11q_3=sum(count_allele11q_3)/(nqueens*queenalleles);
freqallele12q_3=sum(count_allele12q_3)/(nqueens*queenalleles);

freqallele1_3=((2*freqallele1q_3+freqallele1q_2)/3)
freqallele2_3=((2*freqallele2q_3+freqallele2q_2)/3)
freqallele3_3=((2*freqallele3q_3+freqallele3q_2)/3)
freqallele4_3=((2*freqallele4q_3+freqallele4q_2)/3)
freqallele5_3=((2*freqallele5q_3+freqallele5q_2)/3)
freqallele6_3=((2*freqallele6q_3+freqallele6q_2)/3)
freqallele7_3=((2*freqallele7q_3+freqallele7q_2)/3)
freqallele8_3=((2*freqallele8q_3+freqallele8q_2)/3)
freqallele9_3=((2*freqallele9q_3+freqallele9q_2)/3)
freqallele10_3=((2*freqallele10q_3+freqallele10q_2)/3)
freqallele11_3=((2*freqallele11q_3+freqallele11q_2)/3)
freqallele12_3=((2*freqallele12q_3+freqallele12q_2)/3)

mat1_G3(freqallele1)=freqallele1_3
mat2_G3(freqallele1)=freqallele2_3
mat3_G3(freqallele1)=freqallele3_3
mat4_G3(freqallele1)=freqallele4_3
mat5_G3(freqallele1)=freqallele5_3
mat6_G3(freqallele1)=freqallele6_3
mat7_G3(freqallele1)=freqallele7_3
mat8_G3(freqallele1)=freqallele8_3
mat9_G3(freqallele1)=freqallele9_3
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mat10_G3(freqallele1)=freqallele10_3
mat11_G3(freqallele1)=freqallele11_3
mat12_G3(freqallele1)=freqallele12_3


Breeders4=datasample(Distribution,nobreeders4);

%allele frequencies in queen generation (G4)
allele1q_4=(Breeders4>0) & (Breeders4<freqallele1_3);
allele2q_4=(Breeders4>=freqallele1_3) &
(Breeders4<(freqallele1_3+freqallele2_3));
allele3q_4=(Breeders4>=(freqallele1_3+freqallele2_3)) &
(Breeders4<(freqallele1_3+freqallele2_3+freqallele3_3));
allele4q_4=(Breeders4>=(freqallele1_3+freqallele2_3+freqallele3_3)) &
(Breeders4<(freqallele1_3+freqallele2_3+freqallele3_3+freqallele4_3));
allele5q_4=(Breeders4>=(freqallele1_3+freqallele2_3+freqallele3_3+freqalle
le4_3)) &
(Breeders4<(freqallele1_3+freqallele2_3+freqallele3_3+freqallele4_3+freqal
lele5_3));
allele6q_4=(Breeders4>=(freqallele1_3+freqallele2_3+freqallele3_3+freqalle
le4_3+freqallele5_3)) &
(Breeders4<(freqallele1_3+freqallele2_3+freqallele3_3+freqallele4_3+freqal
lele5_3+freqallele6_3));
allele7q_4=(Breeders4>=(freqallele1_3+freqallele2_3+freqallele3_3+freqalle
le4_3+freqallele5_3+freqallele6_3)) &
(Breeders4<(freqallele1_3+freqallele2_3+freqallele3_3+freqallele4_3+freqal
lele5_3+freqallele6_3+freqallele7_3));
allele8q_4=(Breeders4>=(freqallele1_3+freqallele2_3+freqallele3_3+freqalle
le4_3+freqallele5_3+freqallele6_3+freqallele7_3)) &
(Breeders4<(freqallele1_3+freqallele2_3+freqallele3_3+freqallele4_3+freqal
lele5_3+freqallele6_3+freqallele7_3+freqallele8_3));
allele9q_4=(Breeders4>=(freqallele1_3+freqallele2_3+freqallele3_3+freqalle
le4_3+freqallele5_3+freqallele6_3+freqallele7_3+freqallele8_3)) &
(Breeders4<(freqallele1_3+freqallele2_3+freqallele3_3+freqallele4_3+freqal
lele5_3+freqallele6_3+freqallele7_3+freqallele8_3+freqallele9_3));
allele10q_4=(Breeders4>=(freqallele1_3+freqallele2_3+freqallele3_3+freqall
ele4_3+freqallele5_3+freqallele6_3+freqallele7_3+freqallele8_3+freqallele9
_3)) &
(Breeders4<(freqallele1_3+freqallele2_3+freqallele3_3+freqallele4_3+freqal
lele5_3+freqallele6_3+freqallele7_3+freqallele8_3+freqallele9_3+freqallele
10_3));
allele11q_4=(Breeders4>=(freqallele1_3+freqallele2_3+freqallele3_3+freqall
ele4_3+freqallele5_3+freqallele6_3+freqallele7_3+freqallele8_3+freqallele9
_3+freqallele10_3)) &
(Breeders4<(freqallele1_3+freqallele2_3+freqallele3_3+freqallele4_3+freqal
lele5_3+freqallele6_3+freqallele7_3+freqallele8_3+freqallele9_3+freqallele
10_3+freqallele11_3));
allele12q_4=(Breeders4>=(freqallele1_3+freqallele2_3+freqallele3_3+freqall
ele4_3+freqallele5_3+freqallele6_3+freqallele7_3+freqallele8_3+freqallele9
_3+freqallele10_3+freqallele11_3)) & (Breeders4<1.0);


count_allele1q_4=sum(allele1q_4);
count_allele2q_4=sum(allele2q_4);
```

```
count_allele3q_4=sum(allele3q_4);
count_allele4q_4=sum(allele4q_4);
count_allele5q_4=sum(allele5q_4);
count_allele6q_4=sum(allele6q_4);
count_allele7q_4=sum(allele7q_4);
count_allele8q_4=sum(allele8q_4);
count_allele9q_4=sum(allele9q_4);
count_allele10q_4=sum(allele10q_4);
count_allele11q_4=sum(allele11q_4);
count_allele12q_4=sum(allele12q_4);

freqallele1q_4=sum(count_allele1q_4)/(nobreeders4*queenalleles);
freqallele2q_4=sum(count_allele2q_4)/(nobreeders4*queenalleles);
freqallele3q_4=sum(count_allele3q_4)/(nobreeders4*queenalleles);
freqallele4q_4=sum(count_allele4q_4)/(nobreeders4*queenalleles);
freqallele5q_4=sum(count_allele5q_4)/(nobreeders4*queenalleles);
freqallele6q_4=sum(count_allele6q_4)/(nobreeders4*queenalleles);
freqallele7q_4=sum(count_allele7q_4)/(nobreeders4*queenalleles);
freqallele8q_4=sum(count_allele8q_4)/(nobreeders4*queenalleles);
freqallele9q_4=sum(count_allele9q_4)/(nobreeders4*queenalleles);
freqallele10q_4=sum(count_allele10q_4)/(nobreeders4*queenalleles);
freqallele11q_4=sum(count_allele11q_4)/(nobreeders4*queenalleles);
freqallele12q_4=sum(count_allele12q_4)/(nobreeders4*queenalleles);

Ballelefreq1_G4=(2*freqallele1q_4+freqallele1q_3)/3
Ballelefreq2_G4=(2*freqallele2q_4+freqallele2q_3)/3
Ballelefreq3_G4=(2*freqallele3q_4+freqallele3q_3)/3
Ballelefreq4_G4=(2*freqallele4q_4+freqallele4q_3)/3
Ballelefreq5_G4=(2*freqallele5q_4+freqallele5q_3)/3
Ballelefreq6_G4=(2*freqallele6q_4+freqallele6q_3)/3
Ballelefreq7_G4=(2*freqallele7q_4+freqallele7q_3)/3
Ballelefreq8_G4=(2*freqallele8q_4+freqallele8q_3)/3
Ballelefreq9_G4=(2*freqallele9q_4+freqallele9q_3)/3
Ballelefreq10_G4=(2*freqallele10q_4+freqallele10q_3)/3
Ballelefreq11_G4=(2*freqallele11q_4+freqallele11q_3)/3
Ballelefreq12_G4=(2*freqallele12q_4+freqallele12q_3)/3

%GENERATE 100 QUEENS
DistGen4Q=rand(nqueens,queenalleles);

allele1q_4=(DistGen4Q>0) & (DistGen4Q<Ballelefreq1_G4);
allele2q_4=(DistGen4Q>=Ballelefreq1_G4) &
(DistGen4Q<(Ballelefreq1_G4+Ballelefreq2_G4));
allele3q_4=(DistGen4Q>=(Ballelefreq1_G4+Ballelefreq2_G4)) &
(DistGen4Q<(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4));
allele4q_4=(DistGen4Q>=(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4))
&
(DistGen4Q<(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ballelefreq4_G
4));
allele5q_4=(DistGen4Q>=(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ba
llelefreq4_G4)) &
(DistGen4Q<(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ballelefreq4_G
4+Ballelefreq5_G4));
```

```
allele6q_4=(DistGen4Q>=(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ba
llelefreq4_G4+Ballelefreq5_G4)) &
(DistGen4Q<(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ballelefreq4_G
4+Ballelefreq5_G4+Ballelefreq6_G4));
allele7q_4=(DistGen4Q>=(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ba
llelefreq4_G4+Ballelefreq5_G4+Ballelefreq6_G4)) &
(DistGen4Q<(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ballelefreq4_G
4+Ballelefreq5_G4+Ballelefreq6_G4+Ballelefreq7_G4));
allele8q_4=(DistGen4Q>=(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ba
llelefreq4_G4+Ballelefreq5_G4+Ballelefreq6_G4+Ballelefreq7_G4)) &
(DistGen4Q<(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ballelefreq4_G
4+Ballelefreq5_G4+Ballelefreq6_G4+Ballelefreq7_G4+Ballelefreq8_G4));
allele9q_4=(DistGen4Q>=(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ba
llelefreq4_G4+Ballelefreq5_G4+Ballelefreq6_G4+Ballelefreq7_G4+Ballelefreq8
_G4)) &
(DistGen4Q<(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ballelefreq4_G
4+Ballelefreq5_G4+Ballelefreq6_G4+Ballelefreq7_G4+Ballelefreq8_G4+Ballelef
req9_G4));
allele10q_4=(DistGen4Q>=(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+B
allelefreq4_G4+Ballelefreq5_G4+Ballelefreq6_G4+Ballelefreq7_G4+Ballelefreq
8_G4+Ballelefreq9_G4)) &
(DistGen4Q<(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ballelefreq4_G
4+Ballelefreq5_G4+Ballelefreq6_G4+Ballelefreq7_G4+Ballelefreq8_G4+Ballelef
req9_G4+Ballelefreq10_G4));
allele11q_4=(DistGen4Q>=(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+B
allelefreq4_G4+Ballelefreq5_G4+Ballelefreq6_G4+Ballelefreq7_G4+Ballelefreq
8_G4+Ballelefreq9_G4+Ballelefreq10_G4)) &
(DistGen4Q<(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+Ballelefreq4_G
4+Ballelefreq5_G4+Ballelefreq6_G4+Ballelefreq7_G4+Ballelefreq8_G4+Ballelef
req9_G4+Ballelefreq10_G4+Ballelefreq11_G4));
allele12q_4=(DistGen4Q>=(Ballelefreq1_G4+Ballelefreq2_G4+Ballelefreq3_G4+B
allelefreq4_G4+Ballelefreq5_G4+Ballelefreq6_G4+Ballelefreq7_G4+Ballelefreq
8_G4+Ballelefreq9_G4+Ballelefreq10_G4+Ballelefreq11_G4)) & (DistGen4Q<1);

count_allele1q_4=sum(allele1q_4);
count_allele2q_4=sum(allele2q_4);
count_allele3q_4=sum(allele3q_4);
count_allele4q_4=sum(allele4q_4);
count_allele5q_4=sum(allele5q_4);
count_allele6q_4=sum(allele6q_4);
count_allele7q_4=sum(allele7q_4);
count_allele8q_4=sum(allele8q_4);
count_allele9q_4=sum(allele9q_4);
count_allele10q_4=sum(allele10q_4);
count_allele11q_4=sum(allele11q_4);
count_allele12q_4=sum(allele12q_4);

freqallele1q_4=sum(count_allele1q_4)/(nqueens*queenalleles);
freqallele2q_4=sum(count_allele2q_4)/(nqueens*queenalleles);
freqallele3q_4=sum(count_allele3q_4)/(nqueens*queenalleles);
freqallele4q_4=sum(count_allele4q_4)/(nqueens*queenalleles);
freqallele5q_4=sum(count_allele5q_4)/(nqueens*queenalleles);
freqallele6q_4=sum(count_allele6q_4)/(nqueens*queenalleles);
freqallele7q_4=sum(count_allele7q_4)/(nqueens*queenalleles);
freqallele8q_4=sum(count_allele8q_4)/(nqueens*queenalleles);
```

```
freqallele9q_4=sum(count_allele9q_4)/(nqueens*queenalleles);
freqallele10q_4=sum(count_allele10q_4)/(nqueens*queenalleles);
freqallele11q_4=sum(count_allele11q_4)/(nqueens*queenalleles);
freqallele12q_4=sum(count_allele12q_4)/(nqueens*queenalleles);

freqallele1_4=((2*freqallele1q_4+freqallele1q_3)/3)
freqallele2_4=((2*freqallele2q_4+freqallele2q_3)/3)
freqallele3_4=((2*freqallele3q_4+freqallele3q_3)/3)
freqallele4_4=((2*freqallele4q_4+freqallele4q_3)/3)
freqallele5_4=((2*freqallele5q_4+freqallele5q_3)/3)
freqallele6_4=((2*freqallele6q_4+freqallele6q_3)/3)
freqallele7_4=((2*freqallele7q_4+freqallele7q_3)/3)
freqallele8_4=((2*freqallele8q_4+freqallele8q_3)/3)
freqallele9_4=((2*freqallele9q_4+freqallele9q_3)/3)
freqallele10_4=((2*freqallele10q_4+freqallele10q_3)/3)
freqallele11_4=((2*freqallele11q_4+freqallele11q_3)/3)
freqallele12_4=((2*freqallele12q_4+freqallele12q_3)/3)

mat1_G4(freqallele1)=freqallele1_4
mat2_G4(freqallele1)=freqallele2_4
mat3_G4(freqallele1)=freqallele3_4
mat4_G4(freqallele1)=freqallele4_4
mat5_G4(freqallele1)=freqallele5_4
mat6_G4(freqallele1)=freqallele6_4
mat7_G4(freqallele1)=freqallele7_4
mat8_G4(freqallele1)=freqallele8_4
mat9_G4(freqallele1)=freqallele9_4
mat10_G4(freqallele1)=freqallele10_4
mat11_G4(freqallele1)=freqallele11_4
mat12_G4(freqallele1)=freqallele12_4


Breeders5=datasample(Distribution,nobreeders5);

%allele frequencies in queen generation (G5)



allele1q_5=(Breeders5>0) & (Breeders5<freqallele1_4);
allele2q_5=(Breeders5>=freqallele1_4) &
(Breeders5<(freqallele1_4+freqallele2_4));
allele3q_5=(Breeders5>=(freqallele1_4+freqallele2_4)) &
(Breeders5<(freqallele1_4+freqallele2_4+freqallele3_4));
allele4q_5=(Breeders5>=(freqallele1_4+freqallele2_4+freqallele3_4)) &
(Breeders5<(freqallele1_4+freqallele2_4+freqallele3_4+freqallele4_4));
allele5q_5=(Breeders5>=(freqallele1_4+freqallele2_4+freqallele3_4+freqalle
le4_4)) &
(Breeders5<(freqallele1_4+freqallele2_4+freqallele3_4+freqallele4_4+freqal
lele5_4));
allele6q_5=(Breeders5>=(freqallele1_4+freqallele2_4+freqallele3_4+freqalle
le4_4+freqallele5_4)) &
(Breeders5<(freqallele1_4+freqallele2_4+freqallele3_4+freqallele4_4+freqal
lele5_4+freqallele6_4));
```

```
allele7q_5=(Breeders5>=(freqallele1_4+freqallele2_4+freqallele3_4+freqalle
le4_4+freqallele5_4+freqallele6_4)) &
(Breeders5<(freqallele1_4+freqallele2_4+freqallele3_4+freqallele4_4+freqal
lele5_4+freqallele6_4+freqallele7_4));
allele8q_5=(Breeders5>=(freqallele1_4+freqallele2_4+freqallele3_4+freqalle
le4_4+freqallele5_4+freqallele6_4+freqallele7_4)) &
(Breeders5<(freqallele1_4+freqallele2_4+freqallele3_4+freqallele4_4+freqal
lele5_4+freqallele6_4+freqallele7_4+freqallele8_4));
allele9q_5=(Breeders5>=(freqallele1_4+freqallele2_4+freqallele3_4+freqalle
le4_4+freqallele5_4+freqallele6_4+freqallele7_4+freqallele8_4)) &
(Breeders5<(freqallele1_4+freqallele2_4+freqallele3_4+freqallele4_4+freqal
lele5_4+freqallele6_4+freqallele7_4+freqallele8_4+freqallele9_4));
allele10q_5=(Breeders5>=(freqallele1_4+freqallele2_4+freqallele3_4+freqall
ele4_4+freqallele5_4+freqallele6_4+freqallele7_4+freqallele8_4+freqallele9
_4)) &
(Breeders5<(freqallele1_4+freqallele2_4+freqallele3_4+freqallele4_4+freqal
lele5_4+freqallele6_4+freqallele7_4+freqallele8_4+freqallele9_4+freqallele
10_4));
allele11q_5=(Breeders5>=(freqallele1_4+freqallele2_4+freqallele3_4+freqall
ele4_4+freqallele5_4+freqallele6_4+freqallele7_4+freqallele8_4+freqallele9
_4+freqallele10_4)) &
(Breeders5<(freqallele1_4+freqallele2_4+freqallele3_4+freqallele4_4+freqal
lele5_4+freqallele6_4+freqallele7_4+freqallele8_4+freqallele9_4+freqallele
10_4+freqallele11_4));
allele12q_5=(Breeders5>=(freqallele1_4+freqallele2_4+freqallele3_4+freqall
ele4_4+freqallele5_4+freqallele6_4+freqallele7_4+freqallele8_4+freqallele9
_4+freqallele10_4+freqallele11_4)) & (Breeders5<1.0);


count_allele1q_5=sum(allele1q_5);
count_allele2q_5=sum(allele2q_5);
count_allele3q_5=sum(allele3q_5);
count_allele4q_5=sum(allele4q_5);
count_allele5q_5=sum(allele5q_5);
count_allele6q_5=sum(allele6q_5);
count_allele7q_5=sum(allele7q_5);
count_allele8q_5=sum(allele8q_5);
count_allele9q_5=sum(allele9q_5);
count_allele10q_5=sum(allele10q_5);
count_allele11q_5=sum(allele11q_5);
count_allele12q_5=sum(allele12q_5);

freqallele1q_5=sum(count_allele1q_5)/(nobreeders5*queenalleles);
freqallele2q_5=sum(count_allele2q_5)/(nobreeders5*queenalleles);
freqallele3q_5=sum(count_allele3q_5)/(nobreeders5*queenalleles);
freqallele4q_5=sum(count_allele4q_5)/(nobreeders5*queenalleles);
freqallele5q_5=sum(count_allele5q_5)/(nobreeders5*queenalleles);
freqallele6q_5=sum(count_allele6q_5)/(nobreeders5*queenalleles);
freqallele7q_5=sum(count_allele7q_5)/(nobreeders5*queenalleles);
freqallele8q_5=sum(count_allele8q_5)/(nobreeders5*queenalleles);
freqallele9q_5=sum(count_allele9q_5)/(nobreeders5*queenalleles);
freqallele10q_5=sum(count_allele10q_5)/(nobreeders5*queenalleles);
freqallele11q_5=sum(count_allele11q_5)/(nobreeders5*queenalleles);
freqallele12q_5=sum(count_allele12q_5)/(nobreeders5*queenalleles);
```

```
Ballelefreq1_G5=(2*freqallele1q_5+freqallele1q_4)/3
Ballelefreq2_G5=(2*freqallele2q_5+freqallele2q_4)/3
Ballelefreq3_G5=(2*freqallele3q_5+freqallele3q_4)/3
Ballelefreq4_G5=(2*freqallele4q_5+freqallele4q_4)/3
Ballelefreq5_G5=(2*freqallele5q_5+freqallele5q_4)/3
Ballelefreq6_G5=(2*freqallele6q_5+freqallele6q_4)/3
Ballelefreq7_G5=(2*freqallele7q_5+freqallele7q_4)/3
Ballelefreq8_G5=(2*freqallele8q_5+freqallele8q_4)/3
Ballelefreq9_G5=(2*freqallele9q_5+freqallele9q_4)/3
Ballelefreq10_G5=(2*freqallele10q_5+freqallele10q_4)/3
Ballelefreq11_G5=(2*freqallele11q_5+freqallele11q_4)/3
Ballelefreq12_G5=(2*freqallele12q_5+freqallele12q_4)/3

%GENERATE 100 QUEENS
DistGen5Q=rand(nqueens,queenalleles);

allele1q_5=(DistGen5Q>0) & (DistGen5Q<Ballelefreq1_G5);
allele2q_5=(DistGen5Q>=Ballelefreq1_G5) &
(DistGen5Q<(Ballelefreq1_G5+Ballelefreq2_G5));
allele3q_5=(DistGen5Q>=(Ballelefreq1_G5+Ballelefreq2_G5)) &
(DistGen5Q<(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5));
allele4q_5=(DistGen5Q>=(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5))
&
(DistGen5Q<(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ballelefreq4_G
5));
allele5q_5=(DistGen5Q>=(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ba
llelefreq4_G5)) &
(DistGen5Q<(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ballelefreq4_G
5+Ballelefreq5_G5));
allele6q_5=(DistGen5Q>=(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ba
llelefreq4_G5+Ballelefreq5_G5)) &
(DistGen5Q<(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ballelefreq4_G
5+Ballelefreq5_G5+Ballelefreq6_G5));
allele7q_5=(DistGen5Q>=(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ba
llelefreq4_G5+Ballelefreq5_G5+Ballelefreq6_G5)) &
(DistGen5Q<(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ballelefreq4_G
5+Ballelefreq5_G5+Ballelefreq6_G5+Ballelefreq7_G5));
allele8q_5=(DistGen5Q>=(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ba
llelefreq4_G5+Ballelefreq5_G5+Ballelefreq6_G5+Ballelefreq7_G5)) &
(DistGen5Q<(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ballelefreq4_G
5+Ballelefreq5_G5+Ballelefreq6_G5+Ballelefreq7_G5+Ballelefreq8_G5));
allele9q_5=(DistGen5Q>=(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ba
llelefreq4_G5+Ballelefreq5_G5+Ballelefreq6_G5+Ballelefreq7_G5+Ballelefreq8
_G5)) &
(DistGen5Q<(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ballelefreq4_G
5+Ballelefreq5_G5+Ballelefreq6_G5+Ballelefreq7_G5+Ballelefreq8_G5+Ballelef
req9_G5));
allele10q_5=(DistGen5Q>=(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+B
allelefreq4_G5+Ballelefreq5_G5+Ballelefreq6_G5+Ballelefreq7_G5+Ballelefreq
8_G5+Ballelefreq9_G5)) &
(DistGen5Q<(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ballelefreq4_G
5+Ballelefreq5_G5+Ballelefreq6_G5+Ballelefreq7_G5+Ballelefreq8_G5+Ballelef
req9_G5+Ballelefreq10_G5));
```

```
allele11q_5=(DistGen5Q>=(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+B
allelefreq4_G5+Ballelefreq5_G5+Ballelefreq6_G5+Ballelefreq7_G5+Ballelefreq
8_G5+Ballelefreq9_G5+Ballelefreq10_G5)) &
(DistGen5Q<(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+Ballelefreq4_G
5+Ballelefreq5_G5+Ballelefreq6_G5+Ballelefreq7_G5+Ballelefreq8_G5+Ballelef
req9_G5+Ballelefreq10_G5+Ballelefreq11_G5));
allele12q_5=(DistGen5Q>=(Ballelefreq1_G5+Ballelefreq2_G5+Ballelefreq3_G5+B
allelefreq4_G5+Ballelefreq5_G5+Ballelefreq6_G5+Ballelefreq7_G5+Ballelefreq
8_G5+Ballelefreq9_G5+Ballelefreq10_G5+Ballelefreq11_G5)) & (DistGen5Q<1);

count_allele1q_5=sum(allele1q_5);
count_allele2q_5=sum(allele2q_5);
count_allele3q_5=sum(allele3q_5);
count_allele4q_5=sum(allele4q_5);
count_allele5q_5=sum(allele5q_5);
count_allele6q_5=sum(allele6q_5);
count_allele7q_5=sum(allele7q_5);
count_allele8q_5=sum(allele8q_5);
count_allele9q_5=sum(allele9q_5);
count_allele10q_5=sum(allele10q_5);
count_allele11q_5=sum(allele11q_5);
count_allele12q_5=sum(allele12q_5);

freqallele1q_5=sum(count_allele1q_5)/(nqueens*queenalleles);
freqallele2q_5=sum(count_allele2q_5)/(nqueens*queenalleles);
freqallele3q_5=sum(count_allele3q_5)/(nqueens*queenalleles);
freqallele4q_5=sum(count_allele4q_5)/(nqueens*queenalleles);
freqallele5q_5=sum(count_allele5q_5)/(nqueens*queenalleles);
freqallele6q_5=sum(count_allele6q_5)/(nqueens*queenalleles);
freqallele7q_5=sum(count_allele7q_5)/(nqueens*queenalleles);
freqallele8q_5=sum(count_allele8q_5)/(nqueens*queenalleles);
freqallele9q_5=sum(count_allele9q_5)/(nqueens*queenalleles);
freqallele10q_5=sum(count_allele10q_5)/(nqueens*queenalleles);
freqallele11q_5=sum(count_allele11q_5)/(nqueens*queenalleles);
freqallele12q_5=sum(count_allele12q_5)/(nqueens*queenalleles);

freqallele1_5=((2*freqallele1q_5+freqallele1q_4)/3)
freqallele2_5=((2*freqallele2q_5+freqallele2q_4)/3)
freqallele3_5=((2*freqallele3q_5+freqallele3q_4)/3)
freqallele4_5=((2*freqallele4q_5+freqallele4q_4)/3)
freqallele5_5=((2*freqallele5q_5+freqallele5q_4)/3)
freqallele6_5=((2*freqallele6q_5+freqallele6q_4)/3)
freqallele7_5=((2*freqallele7q_5+freqallele7q_4)/3)
freqallele8_5=((2*freqallele8q_5+freqallele8q_4)/3)
freqallele9_5=((2*freqallele9q_5+freqallele9q_4)/3)
freqallele10_5=((2*freqallele10q_5+freqallele10q_4)/3)
freqallele11_5=((2*freqallele11q_5+freqallele11q_4)/3)
freqallele12_5=((2*freqallele12q_5+freqallele12q_4)/3)

mat1_G5(freqallele1)=freqallele1_5
mat2_G5(freqallele1)=freqallele2_5
mat3_G5(freqallele1)=freqallele3_5
mat4_G5(freqallele1)=freqallele4_5
mat5_G5(freqallele1)=freqallele5_5
```

```
mat6_G5(freqallele1)=freqallele6_5
mat7_G5(freqallele1)=freqallele7_5
mat8_G5(freqallele1)=freqallele8_5
mat9_G5(freqallele1)=freqallele9_5
mat10_G5(freqallele1)=freqallele10_5
mat11_G5(freqallele1)=freqallele11_5
mat12_G5(freqallele1)=freqallele12_5
end


Matallele1=[mat1_G0; mat1_G1; mat1_G2; mat1_G3; mat1_G4; mat1_G5]
Matallele2=[mat2_G0; mat2_G1; mat2_G2; mat2_G3; mat2_G4; mat2_G5]
Matallele3=[mat3_G0; mat3_G1; mat3_G2; mat3_G3; mat3_G4; mat3_G5]
Matallele4=[mat4_G0; mat4_G1; mat4_G2; mat4_G3; mat4_G4; mat4_G5]
Matallele5=[mat5_G0; mat5_G1; mat5_G2; mat5_G3; mat5_G4; mat5_G5]
Matallele6=[mat6_G0; mat6_G1; mat6_G2; mat6_G3; mat6_G4; mat6_G5]
Matallele7=[mat7_G0; mat7_G1; mat7_G2; mat7_G3; mat7_G4; mat7_G5]
Matallele8=[mat8_G0; mat8_G1; mat8_G2; mat8_G3; mat8_G4; mat8_G5]
Matallele9=[mat9_G0; mat9_G1; mat9_G2; mat9_G3; mat9_G4; mat9_G5]
Matallele10=[mat10_G0; mat10_G1; mat10_G2; mat10_G3; mat10_G4; mat10_G5]
Matallele11=[mat11_G0; mat11_G1; mat11_G2; mat11_G3; mat11_G4; mat11_G5]
Matallele12=[mat12_G0; mat12_G1; mat12_G2; mat12_G3; mat12_G4; mat12_G5]

Alldata=[Matallele1; Matallele2; Matallele3; Matallele4; Matallele5;
Matallele6; Matallele7; Matallele8; Matallele9; Matallele10; Matallele11;
Matallele12]
```

Appendix iv. CSD Simulation model (3 generations only)

```
%input allele frequencies
nqueens = 100;
queenalleles=2;
nobreeders1=8;
nobreeders2=4;
nobreeders3=4 ;
nodrones=7;
a =1000;  %number of iterations
%1 GENERATE A BASELINE DRONE AND QUEEN DERIVED FREQUENCY DIST
for freqallele1=(1:a);
     freqallele2=(1:a) ;
         freqallele3=(1:a) ;
             freqallele4=(1:a) ;
                freqallele5=(1:a) ;
                    freqallele6=(1:a) ;
                        freqallele7=(1:a);
                            freqallele8=(1:a) ;
                                freqallele9=(1:a) ;
                                    freqallele10=(1:a) ;
                                        freqallele11=(1:a);
%G0 DRONES

DistG0drones=rand(nqueens,nodrones);
allele1d_0=(DistG0drones>0) & (DistG0drones<0.038);
allele2d_0=(DistG0drones>=0.038) & (DistG0drones<0.113);
allele3d_0=(DistG0drones>=0.113) & (DistG0drones<0.236);
allele4d_0=(DistG0drones>=0.236) & (DistG0drones<0.311);
allele5d_0=(DistG0drones>=0.311) & (DistG0drones<0.425);
allele6d_0=(DistG0drones>=0.425) & (DistG0drones<0.491);
allele7d_0=(DistG0drones>=0.491) & (DistG0drones<0.774);
allele8d_0=(DistG0drones>=0.774) & (DistG0drones<0.896);
allele9d_0=(DistG0drones>=0.896) & (DistG0drones<0.962);
allele10d_0=(DistG0drones>=0.962) & (DistG0drones<0.991);
allele11d_0=(DistG0drones>=0.991) & (DistG0drones<1);

count_allele1d_0=sum(allele1d_0);
count_allele2d_0=sum(allele2d_0);
count_allele3d_0=sum(allele3d_0);
count_allele4d_0=sum(allele4d_0);
count_allele5d_0=sum(allele5d_0);
count_allele6d_0=sum(allele6d_0);
count_allele7d_0=sum(allele7d_0);
count_allele8d_0=sum(allele8d_0);
count_allele9d_0=sum(allele9d_0);
count_allele10d_0=sum(allele10d_0);
count_allele11d_0=sum(allele11d_0);

freqallele1d_G0=sum(count_allele1d_0)/(nqueens*nodrones);
freqallele2d_G0=sum(count_allele2d_0)/(nqueens*nodrones);
freqallele3d_G0=sum(count_allele3d_0)/(nqueens*nodrones);
freqallele4d_G0=sum(count_allele4d_0)/(nqueens*nodrones);
freqallele5d_G0=sum(count_allele5d_0)/(nqueens*nodrones);
freqallele6d_G0=sum(count_allele6d_0)/(nqueens*nodrones);
```

```
freqallele7d_G0=sum(count_allele7d_0)/(nqueens*nodrones);
freqallele8d_G0=sum(count_allele8d_0)/(nqueens*nodrones);
freqallele9d_G0=sum(count_allele9d_0)/(nqueens*nodrones);
freqallele10d_G0=sum(count_allele10d_0)/(nqueens*nodrones) ;
freqallele11d_G0=sum(count_allele11d_0)/(nqueens*nodrones) ;
%GO QUEENS

DistG0queens=rand(nqueens,2) ;

allele1q_0_mat1=(DistG0queens>0) & (DistG0queens<0.038) ;
allele2q_0_mat1=(DistG0queens>=0.038) & (DistG0queens<0.113) ;
allele3q_0_mat1=(DistG0queens>=0.113) & (DistG0queens<0.236) ;
allele4q_0_mat1=(DistG0queens>=0.236) & (DistG0queens<0.311) ;
allele5q_0_mat1=(DistG0queens>=0.311) & (DistG0queens<0.425) ;
allele6q_0_mat1=(DistG0queens>=0.425) & (DistG0queens<0.491) ;
allele7q_0_mat1=(DistG0queens>=0.491) & (DistG0queens<0.774) ;
allele8q_0_mat1=(DistG0queens>=0.774) & (DistG0queens<0.896) ;
allele9q_0_mat1=(DistG0queens>=0.896) & (DistG0queens<0.962) ;
allele10q_0_mat1=(DistG0queens>=0.962) & (DistG0queens<0.991) ;
allele11q_0_mat1=(DistG0queens>=0.991) & (DistG0queens<1) ;

%DELETES HOMOZYGOTES
allele1q_0_mat1(all(allele1q_0_mat1==1,2),:)=[];
allele2q_0_mat1(all(allele2q_0_mat1==1,2),:)=[];
allele3q_0_mat1(all(allele3q_0_mat1==1,2),:)=[];
allele4q_0_mat1(all(allele4q_0_mat1==1,2),:)=[];
allele5q_0_mat1(all(allele5q_0_mat1==1,2),:)=[];
allele6q_0_mat1(all(allele6q_0_mat1==1,2),:)=[];
allele7q_0_mat1(all(allele7q_0_mat1==1,2),:)=[];
allele8q_0_mat1(all(allele8q_0_mat1==1,2),:)=[];
allele9q_0_mat1(all(allele9q_0_mat1==1,2),:)=[];
allele10q_0_mat1(all(allele10q_0_mat1==1,2),:)=[];
allele11q_0_mat1(all(allele11q_0_mat1==1,2),:)=[];

% HOW MANY ROWS DELETED PER ALLELE CLASS

rows1=nqueens-length(allele1q_0_mat1(:,1));
rows2=nqueens-length(allele2q_0_mat1(:,1));
rows3=nqueens-length(allele3q_0_mat1(:,1));
rows4=nqueens-length(allele4q_0_mat1(:,1));
rows5=nqueens-length(allele5q_0_mat1(:,1));
rows6=nqueens-length(allele6q_0_mat1(:,1));
rows7=nqueens-length(allele7q_0_mat1(:,1));
rows8=nqueens-length(allele8q_0_mat1(:,1));
rows9=nqueens-length(allele9q_0_mat1(:,1));
rows10=nqueens-length(allele10q_0_mat1(:,1));
rows11=nqueens-length(allele11q_0_mat1(:,1));

% TOTAL NUMBER OF ROWS DELETED
G0totalrows1=(rows1+rows2+rows3+rows4+rows5+rows6+rows7+rows8+rows9+rows10
+rows11);

Count_allele1q_0_mat1=sum(allele1q_0_mat1);
Count_allele2q_0_mat1=sum(allele2q_0_mat1);
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```
Count_allele3q_0_mat1=sum(allele3q_0_mat1);
Count_allele4q_0_mat1=sum(allele4q_0_mat1);
Count_allele5q_0_mat1=sum(allele5q_0_mat1);
Count_allele6q_0_mat1=sum(allele6q_0_mat1);
Count_allele7q_0_mat1=sum(allele7q_0_mat1);
Count_allele8q_0_mat1=sum(allele8q_0_mat1);
Count_allele9q_0_mat1=sum(allele9q_0_mat1);
Count_allele10q_0_mat1=sum(allele10q_0_mat1);
Count_allele11q_0_mat1=sum(allele11q_0_mat1);

freqallele1q_0_mat1=sum(Count_allele1q_0_mat1)/(nqueens*2) ;
freqallele2q_0_mat1=sum(Count_allele2q_0_mat1)/(nqueens*2);
freqallele3q_0_mat1=sum(Count_allele3q_0_mat1)/(nqueens*2) ;
freqallele4q_0_mat1=sum(Count_allele4q_0_mat1)/(nqueens*2) ;
freqallele5q_0_mat1=sum(Count_allele5q_0_mat1)/(nqueens*2) ;
freqallele6q_0_mat1=sum(Count_allele6q_0_mat1)/(nqueens*2) ;
freqallele7q_0_mat1=sum(Count_allele7q_0_mat1)/(nqueens*2) ;
freqallele8q_0_mat1=sum(Count_allele8q_0_mat1)/(nqueens*2) ;
freqallele9q_0_mat1=sum(Count_allele9q_0_mat1)/(nqueens*2) ;
freqallele10q_0_mat1=sum(Count_allele10q_0_mat1)/(nqueens*2) ;
freqallele11q_0_mat1=sum(Count_allele11q_0_mat1)/(nqueens*2) ;

%GENERATE NEW ARRAY EQUALIN SIZE TO RELACE REMOVED ROWS ABOVE

dist1= rand(G0totalrows1,2) ;

allele1q_0_mat2=(dist1>0) & (dist1<0.038);
allele2q_0_mat2=(dist1>=0.038) & (dist1<0.113);
allele3q_0_mat2=(dist1>=0.113) & (dist1<0.236);
allele4q_0_mat2=(dist1>=0.236) & (dist1<0.311);
allele5q_0_mat2=(dist1>0.311) & (dist1<0.425) ;
allele6q_0_mat2=(dist1>=0.425) & (dist1<0.491);
allele7q_0_mat2=(dist1>=0.491) & (dist1<0.774);
allele8q_0_mat2=(dist1>=0.774) & (dist1<0.896) ;
allele9q_0_mat2=(dist1>=0.896) & (dist1<0.962);
allele10q_0_mat2=(dist1>=0.962) & (dist1<0.991);
allele11q_0_mat2=(dist1>=0.991) & (dist1<1) ;

%PURGE FOR HOMO AGAIN
allele1q_0_mat2(all(allele1q_0_mat2==1,2),:)=[];
allele2q_0_mat2(all(allele2q_0_mat2==1,2),:)=[] ;
allele3q_0_mat2(all(allele3q_0_mat2==1,2),:)=[] ;
allele4q_0_mat2(all(allele4q_0_mat2==1,2),:)=[] ;
allele5q_0_mat2(all(allele5q_0_mat2==1,2),:)=[];
allele6q_0_mat2(all(allele6q_0_mat2==1,2),:)=[];
allele7q_0_mat2(all(allele7q_0_mat2==1,2),:)=[];
allele8q_0_mat2(all(allele8q_0_mat2==1,2),:)=[];
allele9q_0_mat2(all(allele9q_0_mat2==1,2),:)=[];
allele10q_0_mat2(all(allele10q_0_mat2==1,2),:)=[];
allele11q_0_mat2(all(allele11q_0_mat2==1,2),:)=[];

rows12=G0totalrows1-length(allele1q_0_mat2(:,1));  %allele1 in matrix2
rows13=G0totalrows1-length(allele2q_0_mat2(:,1));  %allele2 in matrix2
rows14=G0totalrows1-length(allele3q_0_mat2(:,1));
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rows15=G0totalrows1-length(allele4q_0_mat2(:,1));
rows16=G0totalrows1-length(allele5q_0_mat2(:,1));
rows17=G0totalrows1-length(allele6q_0_mat2(:,1));
rows18=G0totalrows1-length(allele7q_0_mat2(:,1));
rows19=G0totalrows1-length(allele8q_0_mat2(:,1));
rows20=G0totalrows1-length(allele9q_0_mat2(:,1));
rows21=G0totalrows1-length(allele10q_0_mat2(:,1));
rows22=G0totalrows1-length(allele11q_0_mat2(:,1));

%TOTAL NUMBER OF ALLELES REMOVED FROM MATRIX 2
G0totalrows2=(rows12+rows13+rows14+rows15+rows16+rows17+rows18+rows19+rows
20+rows21+rows22);

Count_allele1q_0_mat2=sum(allele1q_0_mat2) ;
Count_allele2q_0_mat2=sum(allele2q_0_mat2);
Count_allele3q_0_mat2=sum(allele3q_0_mat2);
Count_allele4q_0_mat2=sum(allele4q_0_mat2);
Count_allele5q_0_mat2=sum(allele5q_0_mat2);
Count_allele6q_0_mat2=sum(allele6q_0_mat2) ;
Count_allele7q_0_mat2=sum(allele7q_0_mat2);
Count_allele8q_0_mat2=sum(allele8q_0_mat2);
Count_allele9q_0_mat2=sum(allele9q_0_mat2) ;
Count_allele10q_0_mat2=sum(allele10q_0_mat2);
Count_allele11q_0_mat2=sum(allele11q_0_mat2);

freqallele1q_0_mat2=sum(Count_allele1q_0_mat2)/(nqueens*2);
freqallele2q_0_mat2=sum(Count_allele2q_0_mat2)/(nqueens*2) ;
freqallele3q_0_mat2=sum(Count_allele3q_0_mat2)/(nqueens*2);
freqallele4q_0_mat2=sum(Count_allele4q_0_mat2)/(nqueens*2);
freqallele5q_0_mat2=sum(Count_allele5q_0_mat2)/(nqueens*2);
freqallele6q_0_mat2=sum(Count_allele6q_0_mat2)/(nqueens*2);
freqallele7q_0_mat2=sum(Count_allele7q_0_mat2)/(nqueens*2);
freqallele8q_0_mat2=sum(Count_allele8q_0_mat2)/(nqueens*2);
freqallele9q_0_mat2=sum(Count_allele9q_0_mat2)/(nqueens*2);
freqallele10q_0_mat2=sum(Count_allele10q_0_mat2)/(nqueens*2);
freqallele11q_0_mat2=sum(Count_allele11q_0_mat2)/(nqueens*2);
dist2= rand(G0totalrows2,2) ;

allele1q_0_mat3=(dist2>0) & (dist2<0.038) ;
allele2q_0_mat3=(dist2>=0.038) & (dist2<0.113);
allele3q_0_mat3=(dist2>=0.113) & (dist2<0.236);
allele4q_0_mat3=(dist2>=0.236) & (dist2<0.311);
allele5q_0_mat3=(dist2>0.311) & (dist2<0.425) ;
allele6q_0_mat3=(dist2>=0.425) & (dist2<0.491);
allele7q_0_mat3=(dist2>=0.491) & (dist2<0.774);
allele8q_0_mat3=(dist2>=0.774) & (dist2<0.896) ;
allele9q_0_mat3=(dist2>=0.896) & (dist2<0.962);
allele10q_0_mat3=(dist2>=0.962) & (dist2<0.991);
allele11q_0_mat3=(dist2>=0.991) & (dist2<1) ;

allele1q_0_mat3(all(allele1q_0_mat3==1,2),:)=[] ;
allele2q_0_mat3(all(allele2q_0_mat3==1,2),:)=[] ;
allele3q_0_mat3(all(allele3q_0_mat3==1,2),:)=[] ;
allele4q_0_mat3(all(allele4q_0_mat3==1,2),:)=[] ;
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allele5q_0_mat3(all(allele5q_0_mat3==1,2),:)=[] ;
allele6q_0_mat3(all(allele6q_0_mat3==1,2),:)=[] ;
allele7q_0_mat3(all(allele7q_0_mat3==1,2),:)=[];
allele8q_0_mat3(all(allele8q_0_mat3==1,2),:)=[] ;
allele9q_0_mat3(all(allele9q_0_mat3==1,2),:)=[] ;
allele10q_0_mat3(all(allele10q_0_mat3==1,2),:)=[];
allele11q_0_mat3(all(allele11q_0_mat3==1,2),:)=[] ;

rows23=G0totalrows2-length(allele1q_0_mat3(:,1));
rows24=G0totalrows2-length(allele2q_0_mat3(:,1)) ;
rows25=G0totalrows2-length(allele3q_0_mat3(:,1)) ;
rows26=G0totalrows2-length(allele4q_0_mat3(:,1)) ;
rows27=G0totalrows2-length(allele5q_0_mat3(:,1)) ;
rows28=G0totalrows2-length(allele6q_0_mat3(:,1)) ;
rows29=G0totalrows2-length(allele7q_0_mat3(:,1)) ;
rows30=G0totalrows2-length(allele8q_0_mat3(:,1)) ;
rows31=G0totalrows2-length(allele9q_0_mat3(:,1)) ;
rows32=G0totalrows2-length(allele10q_0_mat3(:,1)) ;
rows33=G0totalrows2-length(allele11q_0_mat3(:,1)) ;

G0totalrows3=(rows23+rows24+rows25+rows26+rows27+rows28+rows29+rows30+rows
31+rows32+rows33);

Count_allele1q_0_mat3=sum(allele1q_0_mat3);
Count_allele2q_0_mat3=sum(allele2q_0_mat3) ;
Count_allele3q_0_mat3=sum(allele3q_0_mat3);
Count_allele4q_0_mat3=sum(allele4q_0_mat3);
Count_allele5q_0_mat3=sum(allele5q_0_mat3) ;
Count_allele6q_0_mat3=sum(allele6q_0_mat3) ;
Count_allele7q_0_mat3=sum(allele7q_0_mat3) ;
Count_allele8q_0_mat3=sum(allele8q_0_mat3) ;
Count_allele9q_0_mat3=sum(allele9q_0_mat3) ;
Count_allele10q_0_mat3=sum(allele10q_0_mat3) ;
Count_allele11q_0_mat3=sum(allele11q_0_mat3) ;

freqallele1q_0_mat3=sum(Count_allele1q_0_mat3)/(nqueens*2);
freqallele2q_0_mat3=sum(Count_allele2q_0_mat3)/(nqueens*2);
freqallele3q_0_mat3=sum(Count_allele3q_0_mat3)/(nqueens*2);
freqallele4q_0_mat3=sum(Count_allele4q_0_mat3)/(nqueens*2);
freqallele5q_0_mat3=sum(Count_allele5q_0_mat3)/(nqueens*2);
freqallele6q_0_mat3=sum(Count_allele6q_0_mat3)/(nqueens*2) ;
freqallele7q_0_mat3=sum(Count_allele7q_0_mat3)/(nqueens*2) ;
freqallele8q_0_mat3=sum(Count_allele8q_0_mat3)/(nqueens*2);
freqallele9q_0_mat3=sum(Count_allele9q_0_mat3)/(nqueens*2) ;
freqallele10q_0_mat3=sum(Count_allele10q_0_mat3)/(nqueens*2) ;
freqallele11q_0_mat3=sum(Count_allele11q_0_mat3)/(nqueens*2);

dist3=rand(G0totalrows3,2);

allele1q_0_mat4=(dist3>0) & (dist3<0.038) ;
allele2q_0_mat4=(dist3>=0.038) & (dist3<0.113) ;
allele3q_0_mat4=(dist3>=0.113) & (dist3<0.236) ;
allele4q_0_mat4=(dist3>=0.236) & (dist3<0.311) ;
allele5q_0_mat4=(dist3>0.311) & (dist3<0.425) ;
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allele6q_0_mat4=(dist3>=0.425) & (dist3<0.491) ;
allele7q_0_mat4=(dist3>=0.491) & (dist3<0.774);
allele8q_0_mat4=(dist3>=0.774) & (dist3<0.896) ;
allele9q_0_mat4=(dist3>=0.896) & (dist3<0.962) ;
allele10q_0_mat4=(dist3>=0.962) & (dist3<0.991);
allele11q_0_mat4=(dist3>=0.991) & (dist3<1) ;

allele1q_0_mat4(all(allele1q_0_mat4==1,2),:)=[] ;
allele2q_0_mat4(all(allele2q_0_mat4==1,2),:)=[] ;
allele3q_0_mat4(all(allele3q_0_mat4==1,2),:)=[];
allele4q_0_mat4(all(allele4q_0_mat4==1,2),:)=[];
allele5q_0_mat4(all(allele5q_0_mat4==1,2),:)=[];
allele6q_0_mat4(all(allele6q_0_mat4==1,2),:)=[];
allele7q_0_mat4(all(allele7q_0_mat4==1,2),:)=[];
allele8q_0_mat4(all(allele8q_0_mat4==1,2),:)=[];
allele9q_0_mat4(all(allele9q_0_mat4==1,2),:)=[];
allele10q_0_mat4(all(allele10q_0_mat4==1,2),:)=[];
allele11q_0_mat4(all(allele11q_0_mat4==1,2),:)=[];

rows34=G0totalrows3-length(allele1q_0_mat4(:,1));
rows35=G0totalrows3-length(allele2q_0_mat4(:,1));
rows36=G0totalrows3-length(allele3q_0_mat4(:,1));
rows37=G0totalrows3-length(allele4q_0_mat4(:,1));
rows38=G0totalrows3-length(allele5q_0_mat4(:,1));
rows39=G0totalrows3-length(allele6q_0_mat4(:,1));
rows40=G0totalrows3-length(allele7q_0_mat4(:,1));
rows41=G0totalrows3-length(allele8q_0_mat4(:,1));
rows42=G0totalrows3-length(allele9q_0_mat4(:,1));
rows43=G0totalrows3-length(allele10q_0_mat4(:,1));
rows44=G0totalrows3-length(allele11q_0_mat4(:,1));

G0totalrows4=(rows34+rows35+rows36+rows37+rows38+rows39+rows40+rows41+rows
42+rows43+rows44);

Count_allele1q_0_mat4=sum(allele1q_0_mat4);
Count_allele2q_0_mat4=sum(allele2q_0_mat4) ;
Count_allele3q_0_mat4=sum(allele3q_0_mat4);
Count_allele4q_0_mat4=sum(allele4q_0_mat4);
Count_allele5q_0_mat4=sum(allele5q_0_mat4) ;
Count_allele6q_0_mat4=sum(allele6q_0_mat4);
Count_allele7q_0_mat4=sum(allele7q_0_mat4);
Count_allele8q_0_mat4=sum(allele8q_0_mat4);
Count_allele9q_0_mat4=sum(allele9q_0_mat4);
Count_allele10q_0_mat4=sum(allele10q_0_mat4);
Count_allele11q_0_mat4=sum(allele11q_0_mat4);

freqallele1q_0_mat4=sum(Count_allele1q_0_mat4)/(nqueens*2) ;
freqallele2q_0_mat4=sum(Count_allele2q_0_mat4)/(nqueens*2);
freqallele3q_0_mat4=sum(Count_allele3q_0_mat4)/(nqueens*2);
freqallele4q_0_mat4=sum(Count_allele4q_0_mat4)/(nqueens*2);
freqallele5q_0_mat4=sum(Count_allele5q_0_mat4)/(nqueens*2);
freqallele6q_0_mat4=sum(Count_allele6q_0_mat4)/(nqueens*2);
freqallele7q_0_mat4=sum(Count_allele7q_0_mat4)/(nqueens*2);
freqallele8q_0_mat4=sum(Count_allele8q_0_mat4)/(nqueens*2);
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freqallele9q_0_mat4=sum(Count_allele9q_0_mat4)/(nqueens*2);
freqallele10q_0_mat4=sum(Count_allele10q_0_mat4)/(nqueens*2);
freqallele11q_0_mat4=sum(Count_allele11q_0_mat4)/(nqueens*2);

dist4=rand(G0totalrows4,2) ;

allele1q_0_mat5=(dist4>0) & (dist4<0.038) ;
allele2q_0_mat5=(dist4>=0.038) & (dist4<0.113) ;
allele3q_0_mat5=(dist4>=0.113) & (dist4<0.236) ;
allele4q_0_mat5=(dist4>=0.236) & (dist4<0.311) ;
allele5q_0_mat5=(dist4>0.311) & (dist4<0.425) ;
allele6q_0_mat5=(dist4>=0.425) & (dist4<0.491) ;
allele7q_0_mat5=(dist4>=0.491) & (dist4<0.774);
allele8q_0_mat5=(dist4>=0.774) & (dist4<0.896) ;
allele9q_0_mat5=(dist4>=0.896) & (dist4<0.962) ;
allele10q_0_mat5=(dist4>=0.962) & (dist4<0.991);
allele11q_0_mat5=(dist4>=0.991) & (dist4<1) ;

allele1q_0_mat5(all(allele1q_0_mat5==1,2),:)=[] ;
allele2q_0_mat5(all(allele2q_0_mat5==1,2),:)=[];
allele3q_0_mat5(all(allele3q_0_mat5==1,2),:)=[];
allele4q_0_mat5(all(allele4q_0_mat5==1,2),:)=[];
allele5q_0_mat5(all(allele5q_0_mat5==1,2),:)=[];
allele6q_0_mat5(all(allele6q_0_mat5==1,2),:)=[];
allele7q_0_mat5(all(allele7q_0_mat5==1,2),:)=[];
allele8q_0_mat5(all(allele8q_0_mat5==1,2),:)=[] ;
allele9q_0_mat5(all(allele9q_0_mat5==1,2),:)=[];
allele10q_0_mat5(all(allele10q_0_mat5==1,2),:)=[];
allele11q_0_mat5(all(allele11q_0_mat5==1,2),:)=[] ;

rows45=G0totalrows4-length(allele1q_0_mat5(:,1)) ;
rows46=G0totalrows4-length(allele2q_0_mat5(:,1)) ;
rows47=G0totalrows4-length(allele3q_0_mat5(:,1)) ;
rows48=G0totalrows4-length(allele4q_0_mat5(:,1)) ;
rows49=G0totalrows4-length(allele5q_0_mat5(:,1));
rows50=G0totalrows4-length(allele6q_0_mat5(:,1));
rows51=G0totalrows4-length(allele7q_0_mat5(:,1)) ;
rows52=G0totalrows4-length(allele8q_0_mat5(:,1)) ;
rows53=G0totalrows4-length(allele9q_0_mat5(:,1));
rows54=G0totalrows4-length(allele10q_0_mat5(:,1)) ;
rows55=G0totalrows4-length(allele11q_0_mat5(:,1)) ;

G0totalrows5=(rows45+rows46+rows47+rows48+rows49+rows50+rows51+rows52+rows
53+rows54+rows55);

Count_allele1q_0_mat5=sum(allele1q_0_mat5) ;
Count_allele2q_0_mat5=sum(allele2q_0_mat5) ;
Count_allele3q_0_mat5=sum(allele3q_0_mat5) ;
Count_allele4q_0_mat5=sum(allele4q_0_mat5) ;
Count_allele5q_0_mat5=sum(allele5q_0_mat5) ;
Count_allele6q_0_mat5=sum(allele6q_0_mat5) ;
Count_allele7q_0_mat5=sum(allele7q_0_mat5) ;
Count_allele8q_0_mat5=sum(allele8q_0_mat5) ;
Count_allele9q_0_mat5=sum(allele9q_0_mat5) ;
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Count_allele10q_0_mat5=sum(allele10q_0_mat5) ;
Count_allele11q_0_mat5=sum(allele11q_0_mat5) ;

freqallele1q_0_mat5=sum(Count_allele1q_0_mat5)/(nqueens*2) ;
freqallele2q_0_mat5=sum(Count_allele2q_0_mat5)/(nqueens*2) ;
freqallele3q_0_mat5=sum(Count_allele3q_0_mat5)/(nqueens*2) ;
freqallele4q_0_mat5=sum(Count_allele4q_0_mat5)/(nqueens*2) ;
freqallele5q_0_mat5=sum(Count_allele5q_0_mat5)/(nqueens*2) ;
freqallele6q_0_mat5=sum(Count_allele6q_0_mat5)/(nqueens*2) ;
freqallele7q_0_mat5=sum(Count_allele7q_0_mat5)/(nqueens*2) ;
freqallele8q_0_mat5=sum(Count_allele8q_0_mat5)/(nqueens*2) ;
freqallele9q_0_mat5=sum(Count_allele9q_0_mat5)/(nqueens*2) ;
freqallele10q_0_mat5=sum(Count_allele10q_0_mat5)/(nqueens*2) ;
freqallele11q_0_mat5=sum(Count_allele11q_0_mat5)/(nqueens*2) ;

freqallele1q_G0=freqallele1q_0_mat1+freqallele1q_0_mat2+freqallele1q_0_mat
3+freqallele1q_0_mat4+freqallele1q_0_mat5;
freqallele2q_G0=freqallele2q_0_mat1+freqallele2q_0_mat2+freqallele2q_0_mat
3+freqallele2q_0_mat4+freqallele2q_0_mat5;
freqallele3q_G0=freqallele3q_0_mat1+freqallele3q_0_mat2+freqallele3q_0_mat
3+freqallele3q_0_mat4+freqallele3q_0_mat5;
freqallele4q_G0=freqallele4q_0_mat1+freqallele4q_0_mat2+freqallele4q_0_mat
3+freqallele4q_0_mat4+freqallele4q_0_mat5;
freqallele5q_G0=freqallele5q_0_mat1+freqallele5q_0_mat2+freqallele5q_0_mat
3+freqallele5q_0_mat4+freqallele5q_0_mat5;
freqallele6q_G0=freqallele6q_0_mat1+freqallele6q_0_mat2+freqallele6q_0_mat
3+freqallele6q_0_mat4+freqallele6q_0_mat5;
freqallele7q_G0=freqallele7q_0_mat1+freqallele7q_0_mat2+freqallele7q_0_mat
3+freqallele7q_0_mat4+freqallele7q_0_mat5;
freqallele8q_G0=freqallele8q_0_mat1+freqallele8q_0_mat2+freqallele8q_0_mat
3+freqallele8q_0_mat4+freqallele8q_0_mat5;
freqallele9q_G0=freqallele9q_0_mat1+freqallele9q_0_mat2+freqallele9q_0_mat
3+freqallele9q_0_mat4+freqallele9q_0_mat5;
freqallele10q_G0=freqallele10q_0_mat1+freqallele10q_0_mat2+freqallele10q_0
_mat3+freqallele10q_0_mat4+freqallele10q_0_mat5;
freqallele11q_G0=freqallele11q_0_mat1+freqallele11q_0_mat2+freqallele11q_0
_mat3+freqallele11q_0_mat4+freqallele11q_0_mat5;

%FREQUENCY OF ALLELES IN SIMULATED GENERTION GO
freqallele1_G0=((2*freqallele1q_G0+freqallele1d_G0)/3);
freqallele2_G0=((2*freqallele2q_G0+freqallele2d_G0)/3);
freqallele3_G0=((2*freqallele3q_G0+freqallele3d_G0)/3);
freqallele4_G0=((2*freqallele4q_G0+freqallele4d_G0)/3);
freqallele5_G0=((2*freqallele5q_G0+freqallele5d_G0)/3);
freqallele6_G0=((2*freqallele6q_G0+freqallele6d_G0)/3);
freqallele7_G0=((2*freqallele7q_G0+freqallele7d_G0)/3);
freqallele8_G0=((2*freqallele8q_G0+freqallele8d_G0)/3);
freqallele9_G0=((2*freqallele9q_G0+freqallele9d_G0)/3);
freqallele10_G0=((2*freqallele10q_G0+freqallele10d_G0)/3);
freqallele11_G0=((2*freqallele11q_G0+freqallele11d_G0)/3);

mat1_G0(freqallele1)=freqallele1_G0;
mat2_G0(freqallele1)=freqallele2_G0;
mat3_G0(freqallele1)=freqallele3_G0;
```

```
mat4_G0(freqallele1)=freqallele4_G0;
mat5_G0(freqallele1)=freqallele5_G0;
mat6_G0(freqallele1)=freqallele6_G0;
mat7_G0(freqallele1)=freqallele7_G0;
mat8_G0(freqallele1)=freqallele8_G0;
mat9_G0(freqallele1)=freqallele9_G0;
mat10_G0(freqallele1)=freqallele10_G0;
mat11_G0(freqallele1)=freqallele11_G0;




%NOW DETERMINE ALLELE FREQ IN SELECTED BREEDERS 1

Distribution=rand(nqueens,2);
Breeders1=datasample(Distribution,nobreeders1);

allele1q_b1_mat1=(Breeders1>0) & (Breeders1<freqallele1_G0);
allele2q_b1_mat1=(Breeders1>=freqallele1_G0) &
(Breeders1<(freqallele1_G0+freqallele2_G0));
allele3q_b1_mat1=(Breeders1>=(freqallele1_G0+freqallele2_G0)) &
(Breeders1<(freqallele1_G0+freqallele2_G0+freqallele3_G0));
allele4q_b1_mat1=(Breeders1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0
)) &
(Breeders1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0));
allele5q_b1_mat1=(Breeders1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0
+freqallele4_G0)) &
(Breeders1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+fr
eqallele5_G0));
allele6q_b1_mat1=(Breeders1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0
+freqallele4_G0+freqallele5_G0)) &
(Breeders1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+fr
eqallele5_G0+freqallele6_G0));
allele7q_b1_mat1=(Breeders1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0
+freqallele4_G0+freqallele5_G0+freqallele6_G0)) &
(Breeders1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+fr
eqallele5_G0+freqallele6_G0+ freqallele7_G0));
allele8q_b1_mat1=(Breeders1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0
+freqallele4_G0+freqallele5_G0+freqallele6_G0+ freqallele7_G0)) &
(Breeders1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+fr
eqallele5_G0+freqallele6_G0+ freqallele7_G0+freqallele8_G0));
allele9q_b1_mat1=(Breeders1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0
+freqallele4_G0+freqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0)) &
(Breeders1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+fr
eqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0));
allele10q_b1_mat1=(Breeders1>=(freqallele1_G0+freqallele2_G0+freqallele3_G
0+freqallele4_G0+freqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0)) &
(Breeders1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+fr
eqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0+freqallele10_G0));
```

```
allele11q_b1_mat1=(Breeders1>=(freqallele1_G0+freqallele2_G0+freqallele3_G
0+freqallele4_G0+freqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0+freqallele10_G0)) &
(Breeders1<1);

%PURGE HOMOZYGOTES SELECTED AT RANDOM
allele1q_b1_mat1(all(allele1q_b1_mat1==1,2),:)=[] ;
allele2q_b1_mat1(all(allele2q_b1_mat1==1,2),:)=[] ;
allele3q_b1_mat1(all(allele3q_b1_mat1==1,2),:)=[] ;
allele4q_b1_mat1(all(allele4q_b1_mat1==1,2),:)=[] ;
allele5q_b1_mat1(all(allele5q_b1_mat1==1,2),:)=[] ;
allele6q_b1_mat1(all(allele6q_b1_mat1==1,2),:)=[] ;
allele7q_b1_mat1(all(allele7q_b1_mat1==1,2),:)=[] ;
allele8q_b1_mat1(all(allele8q_b1_mat1==1,2),:)=[] ;
allele9q_b1_mat1(all(allele9q_b1_mat1==1,2),:)=[] ;
allele10q_b1_mat1(all(allele10q_b1_mat1==1,2),:)=[];
allele11q_b1_mat1(all(allele11q_b1_mat1==1,2),:)=[];


rows1=nobreeders1-length(allele1q_b1_mat1(:,1));
rows2=nobreeders1-length(allele2q_b1_mat1(:,1));
rows3=nobreeders1-length(allele3q_b1_mat1(:,1));
rows4=nobreeders1-length(allele4q_b1_mat1(:,1));
rows5=nobreeders1-length(allele5q_b1_mat1(:,1));
rows6=nobreeders1-length(allele6q_b1_mat1(:,1));
rows7=nobreeders1-length(allele7q_b1_mat1(:,1));
rows8=nobreeders1-length(allele8q_b1_mat1(:,1));
rows9=nobreeders1-length(allele9q_b1_mat1(:,1));
rows10=nobreeders1-length(allele10q_b1_mat1(:,1));
rows11=nobreeders1-length(allele11q_b1_mat1(:,1));

G0totalrowsbreeders1=(rows1+rows2+rows3+rows4+rows5+rows6+rows7+rows8+rows
9+rows10+rows11);

count_allele1q_b1_mat1=sum(allele1q_b1_mat1);
count_allele2q_b1_mat1=sum(allele2q_b1_mat1);
count_allele3q_b1_mat1=sum(allele3q_b1_mat1);
count_allele4q_b1_mat1=sum(allele4q_b1_mat1);
count_allele5q_b1_mat1=sum(allele5q_b1_mat1);
count_allele6q_b1_mat1=sum(allele6q_b1_mat1);
count_allele7q_b1_mat1=sum(allele7q_b1_mat1);
count_allele8q_b1_mat1=sum(allele8q_b1_mat1);
count_allele9q_b1_mat1=sum(allele9q_b1_mat1);
count_allele10q_b1_mat1=sum(allele10q_b1_mat1);
count_allele11q_b1_mat1=sum(allele11q_b1_mat1);

freqallele1q_b1_mat1=sum(count_allele1q_b1_mat1)/(nobreeders1*2);
freqallele2q_b1_mat1=sum(count_allele2q_b1_mat1)/(nobreeders1*2);
freqallele3q_b1_mat1=sum(count_allele3q_b1_mat1)/(nobreeders1*2);
freqallele4q_b1_mat1=sum(count_allele4q_b1_mat1)/(nobreeders1*2);
freqallele5q_b1_mat1=sum(count_allele5q_b1_mat1)/(nobreeders1*2);
freqallele6q_b1_mat1=sum(count_allele6q_b1_mat1)/(nobreeders1*2);
freqallele7q_b1_mat1=sum(count_allele7q_b1_mat1)/(nobreeders1*2);
freqallele8q_b1_mat1=sum(count_allele8q_b1_mat1)/(nobreeders1*2);
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freqallele9q_b1_mat1=sum(count_allele9q_b1_mat1)/(nobreeders1*2);
freqallele10q_b1_mat1=sum(count_allele10q_b1_mat1)/(nobreeders1*2);
freqallele11q_b1_mat1=sum(count_allele11q_b1_mat1)/(nobreeders1*2);

dist1=rand(G0totalrowsbreeders1,2);

allele1q_b1_mat2=(dist1>0) & (dist1<freqallele1_G0);
allele2q_b1_mat2=(dist1>=freqallele1_G0) &
(dist1<(freqallele1_G0+freqallele2_G0));
allele3q_b1_mat2=(dist1>=(freqallele1_G0+freqallele2_G0)) &
(dist1<(freqallele1_G0+freqallele2_G0+freqallele3_G0));
allele4q_b1_mat2=(dist1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0)) &
(dist1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0));
allele5q_b1_mat2=(dist1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0)) &
(dist1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0));
allele6q_b1_mat2=(dist1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0)) &
(dist1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0));
allele7q_b1_mat2=(dist1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0+freqallele6_G0)) &
(dist1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+ freqallele7_G0));
allele8q_b1_mat2=(dist1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0+freqallele6_G0+ freqallele7_G0)) &
(dist1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+ freqallele7_G0+freqallele8_G0));
allele9q_b1_mat2=(dist1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0+freqallele6_G0+ freqallele7_G0+freqallele8_G0))
&
(dist1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+ freqallele7_G0+freqallele8_G0+freqallele9_G0));
allele10q_b1_mat2=(dist1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fr
eqallele4_G0+freqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0)) &
(dist1<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0+freqallele10_G0));
allele11q_b1_mat2=(dist1>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fr
eqallele4_G0+freqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0+freqallele10_G0)) &
(dist1<1);

allele1q_b1_mat2(all(allele1q_b1_mat2==1,2),:)=[] ;
allele2q_b1_mat2(all(allele2q_b1_mat2==1,2),:)=[] ;
allele3q_b1_mat2(all(allele3q_b1_mat2==1,2),:)=[] ;
allele4q_b1_mat2(all(allele4q_b1_mat2==1,2),:)=[] ;
allele5q_b1_mat2(all(allele5q_b1_mat2==1,2),:)=[] ;
allele6q_b1_mat2(all(allele6q_b1_mat2==1,2),:)=[] ;
allele7q_b1_mat2(all(allele7q_b1_mat2==1,2),:)=[] ;
allele8q_b1_mat2(all(allele8q_b1_mat2==1,2),:)=[] ;
allele9q_b1_mat2(all(allele9q_b1_mat2==1,2),:)=[] ;
allele10q_b1_mat2(all(allele10q_b1_mat2==1,2),:)=[] ;
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```
allele11q_b1_mat2(all(allele11q_b1_mat2==1,2),:)=[] ;

rows12=G0totalrowsbreeders1-length(allele1q_b1_mat2(:,1));
rows13=G0totalrowsbreeders1-length(allele2q_b1_mat2(:,1));
rows14=G0totalrowsbreeders1-length(allele3q_b1_mat2(:,1));
rows15=G0totalrowsbreeders1-length(allele4q_b1_mat2(:,1));
rows16=G0totalrowsbreeders1-length(allele5q_b1_mat2(:,1));
rows17=G0totalrowsbreeders1-length(allele6q_b1_mat2(:,1));
rows18=G0totalrowsbreeders1-length(allele7q_b1_mat2(:,1));
rows19=G0totalrowsbreeders1-length(allele8q_b1_mat2(:,1));
rows20=G0totalrowsbreeders1-length(allele9q_b1_mat2(:,1));
rows21=G0totalrowsbreeders1-length(allele10q_b1_mat2(:,1));
rows22=G0totalrowsbreeders1-length(allele11q_b1_mat2(:,1));

G0totalrowsbreeders2=(rows12+rows13+rows14+rows15+rows16+rows17+rows18+row
s19+rows20+rows21+rows22);

count_allele1q_b1_mat2=sum(allele1q_b1_mat2);
count_allele2q_b1_mat2=sum(allele2q_b1_mat2);
count_allele3q_b1_mat2=sum(allele3q_b1_mat2);
count_allele4q_b1_mat2=sum(allele4q_b1_mat2);
count_allele5q_b1_mat2=sum(allele5q_b1_mat2);
count_allele6q_b1_mat2=sum(allele6q_b1_mat2);
count_allele7q_b1_mat2=sum(allele7q_b1_mat2);
count_allele8q_b1_mat2=sum(allele8q_b1_mat2);
count_allele9q_b1_mat2=sum(allele9q_b1_mat2);
count_allele10q_b1_mat2=sum(allele10q_b1_mat2);
count_allele11q_b1_mat2=sum(allele11q_b1_mat2);

freqallele1q_b1_mat2=sum(count_allele1q_b1_mat2)/(nobreeders1*2);
freqallele2q_b1_mat2=sum(count_allele2q_b1_mat2)/(nobreeders1*2);
freqallele3q_b1_mat2=sum(count_allele3q_b1_mat2)/(nobreeders1*2);
freqallele4q_b1_mat2=sum(count_allele4q_b1_mat2)/(nobreeders1*2);
freqallele5q_b1_mat2=sum(count_allele5q_b1_mat2)/(nobreeders1*2);
freqallele6q_b1_mat2=sum(count_allele6q_b1_mat2)/(nobreeders1*2);
freqallele7q_b1_mat2=sum(count_allele7q_b1_mat2)/(nobreeders1*2);
freqallele8q_b1_mat2=sum(count_allele8q_b1_mat2)/(nobreeders1*2);
freqallele9q_b1_mat2=sum(count_allele9q_b1_mat2)/(nobreeders1*2);
freqallele10q_b1_mat2=sum(count_allele10q_b1_mat2)/(nobreeders1*2);
freqallele11q_b1_mat2=sum(count_allele11q_b1_mat2)/(nobreeders1*2);

dist2=rand(G0totalrowsbreeders2,2);

allele1q_b1_mat3=(dist2>0) & (dist2<freqallele1_G0);
allele2q_b1_mat3=(dist2>=freqallele1_G0) &
(dist2<(freqallele1_G0+freqallele2_G0));
allele3q_b1_mat3=(dist2>=(freqallele1_G0+freqallele2_G0)) &
(dist2<(freqallele1_G0+freqallele2_G0+freqallele3_G0));
allele4q_b1_mat3=(dist2>=(freqallele1_G0+freqallele2_G0+freqallele3_G0)) &
(dist2<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0));
allele5q_b1_mat3=(dist2>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0)) &
(dist2<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0));
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```
allele6q_b1_mat3=(dist2>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0)) &
(dist2<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0));
allele7q_b1_mat3=(dist2>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0+freqallele6_G0)) &
(dist2<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+ freqallele7_G0));
allele8q_b1_mat3=(dist2>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0+freqallele6_G0+ freqallele7_G0)) &
(dist2<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+ freqallele7_G0+freqallele8_G0));
allele9q_b1_mat3=(dist2>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0+freqallele6_G0+ freqallele7_G0+freqallele8_G0))
&
(dist2<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+ freqallele7_G0+freqallele8_G0+freqallele9_G0));
allele10q_b1_mat3=(dist2>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fr
eqallele4_G0+freqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0)) &
(dist2<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0+freqallele10_G0));
allele11q_b1_mat3=(dist2>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fr
eqallele4_G0+freqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0+freqallele10_G0)) &
(dist2<1);

allele1q_b1_mat3(all(allele1q_b1_mat3==1,2),:)=[] ;
allele2q_b1_mat3(all(allele2q_b1_mat3==1,2),:)=[] ;
allele3q_b1_mat3(all(allele3q_b1_mat3==1,2),:)=[] ;
allele4q_b1_mat3(all(allele4q_b1_mat3==1,2),:)=[] ;
allele5q_b1_mat3(all(allele5q_b1_mat3==1,2),:)=[] ;
allele6q_b1_mat3(all(allele6q_b1_mat3==1,2),:)=[] ;
allele7q_b1_mat3(all(allele7q_b1_mat3==1,2),:)=[] ;
allele8q_b1_mat3(all(allele8q_b1_mat3==1,2),:)=[] ;
allele9q_b1_mat3(all(allele9q_b1_mat3==1,2),:)=[] ;
allele10q_b1_mat3(all(allele10q_b1_mat3==1,2),:)=[] ;
allele11q_b1_mat3(all(allele11q_b1_mat3==1,2),:)=[] ;

rows23=G0totalrowsbreeders2-length(allele1q_b1_mat3(:,1)) ;
rows24=G0totalrowsbreeders2-length(allele2q_b1_mat3(:,1)) ;
rows25=G0totalrowsbreeders2-length(allele3q_b1_mat3(:,1)) ;
rows26=G0totalrowsbreeders2-length(allele4q_b1_mat3(:,1)) ;
rows27=G0totalrowsbreeders2-length(allele5q_b1_mat3(:,1)) ;
rows28=G0totalrowsbreeders2-length(allele6q_b1_mat3(:,1)) ;
rows29=G0totalrowsbreeders2-length(allele7q_b1_mat3(:,1)) ;
rows30=G0totalrowsbreeders2-length(allele8q_b1_mat3(:,1)) ;
rows31=G0totalrowsbreeders2-length(allele9q_b1_mat3(:,1)) ;
rows32=G0totalrowsbreeders2-length(allele10q_b1_mat3(:,1)) ;
rows33=G0totalrowsbreeders2-length(allele11q_b1_mat3(:,1)) ;

G0totalrowsbreeders3=(rows23+rows24+rows25+rows26+rows27+rows28+rows29+row
s30+rows31+rows32+rows33);
```

```
count_allele1q_b1_mat3=sum(allele1q_b1_mat3) ;
count_allele2q_b1_mat3=sum(allele2q_b1_mat3) ;
count_allele3q_b1_mat3=sum(allele3q_b1_mat3) ;
count_allele4q_b1_mat3=sum(allele4q_b1_mat3) ;
count_allele5q_b1_mat3=sum(allele5q_b1_mat3) ;
count_allele6q_b1_mat3=sum(allele6q_b1_mat3) ;
count_allele7q_b1_mat3=sum(allele7q_b1_mat3) ;
count_allele8q_b1_mat3=sum(allele8q_b1_mat3) ;
count_allele9q_b1_mat3=sum(allele9q_b1_mat3) ;
count_allele10q_b1_mat3=sum(allele10q_b1_mat3) ;
count_allele11q_b1_mat3=sum(allele11q_b1_mat3) ;

freqallele1q_b1_mat3=sum(count_allele1q_b1_mat3)/(nobreeders1*2) ;
freqallele2q_b1_mat3=sum(count_allele2q_b1_mat3)/(nobreeders1*2) ;
freqallele3q_b1_mat3=sum(count_allele3q_b1_mat3)/(nobreeders1*2) ;
freqallele4q_b1_mat3=sum(count_allele4q_b1_mat3)/(nobreeders1*2) ;
freqallele5q_b1_mat3=sum(count_allele5q_b1_mat3)/(nobreeders1*2) ;
freqallele6q_b1_mat3=sum(count_allele6q_b1_mat3)/(nobreeders1*2) ;
freqallele7q_b1_mat3=sum(count_allele7q_b1_mat3)/(nobreeders1*2) ;
freqallele8q_b1_mat3=sum(count_allele8q_b1_mat3)/(nobreeders1*2) ;
freqallele9q_b1_mat3=sum(count_allele9q_b1_mat3)/(nobreeders1*2) ;
freqallele10q_b1_mat3=sum(count_allele10q_b1_mat3)/(nobreeders1*2) ;
freqallele11q_b1_mat3=sum(count_allele11q_b1_mat3)/(nobreeders1*2) ;

dist3=rand(G0totalrowsbreeders3,2);

allele1q_b1_mat4=(dist3>0) & (dist3<freqallele1_G0);
allele2q_b1_mat4=(dist3>=freqallele1_G0) &
(dist3<(freqallele1_G0+freqallele2_G0));
allele3q_b1_mat4=(dist3>=(freqallele1_G0+freqallele2_G0)) &
(dist3<(freqallele1_G0+freqallele2_G0+freqallele3_G0));
allele4q_b1_mat4=(dist3>=(freqallele1_G0+freqallele2_G0+freqallele3_G0)) &
(dist3<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0));
allele5q_b1_mat4=(dist3>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0)) &
(dist3<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0));
allele6q_b1_mat4=(dist3>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0)) &
(dist3<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0));
allele7q_b1_mat4=(dist3>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0+freqallele6_G0)) &
(dist3<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+ freqallele7_G0));
allele8q_b1_mat4=(dist3>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0+freqallele6_G0+ freqallele7_G0)) &
(dist3<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+ freqallele7_G0+freqallele8_G0));
allele9q_b1_mat4=(dist3>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fre
qallele4_G0+freqallele5_G0+freqallele6_G0+ freqallele7_G0+freqallele8_G0))
&
```

```
(dist3<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+ freqallele7_G0+freqallele8_G0+freqallele9_G0));
allele10q_b1_mat4=(dist3>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fr
eqallele4_G0+freqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0)) &
(dist3<(freqallele1_G0+freqallele2_G0+freqallele3_G0+freqallele4_G0+freqal
lele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0+freqallele10_G0));
allele11q_b1_mat4=(dist3>=(freqallele1_G0+freqallele2_G0+freqallele3_G0+fr
eqallele4_G0+freqallele5_G0+freqallele6_G0+
freqallele7_G0+freqallele8_G0+freqallele9_G0+freqallele10_G0)) &
(dist3<1);

allele1q_b1_mat4(all(allele1q_b1_mat4==1,2),:)=[] ;
allele2q_b1_mat4(all(allele2q_b1_mat4==1,2),:)=[] ;
allele3q_b1_mat4(all(allele3q_b1_mat4==1,2),:)=[] ;
allele4q_b1_mat4(all(allele4q_b1_mat4==1,2),:)=[] ;
allele5q_b1_mat4(all(allele5q_b1_mat4==1,2),:)=[] ;
allele6q_b1_mat4(all(allele6q_b1_mat4==1,2),:)=[] ;
allele7q_b1_mat4(all(allele7q_b1_mat4==1,2),:)=[] ;
allele8q_b1_mat4(all(allele8q_b1_mat4==1,2),:)=[] ;
allele9q_b1_mat4(all(allele9q_b1_mat4==1,2),:)=[] ;
allele10q_b1_mat4(all(allele10q_b1_mat4==1,2),:)=[] ;
allele11q_b1_mat4(all(allele11q_b1_mat4==1,2),:)=[] ;

rows34=G0totalrowsbreeders3-length(allele1q_b1_mat4(:,1)) ;
rows35=G0totalrowsbreeders3-length(allele2q_b1_mat4(:,1)) ;
rows36=G0totalrowsbreeders3-length(allele3q_b1_mat4(:,1)) ;
rows37=G0totalrowsbreeders3-length(allele4q_b1_mat4(:,1)) ;
rows38=G0totalrowsbreeders3-length(allele5q_b1_mat4(:,1)) ;
rows39=G0totalrowsbreeders3-length(allele6q_b1_mat4(:,1)) ;
rows40=G0totalrowsbreeders3-length(allele7q_b1_mat4(:,1)) ;
rows41=G0totalrowsbreeders3-length(allele8q_b1_mat4(:,1)) ;
rows42=G0totalrowsbreeders3-length(allele9q_b1_mat4(:,1)) ;
rows43=G0totalrowsbreeders3-length(allele10q_b1_mat4(:,1)) ;
rows44=G0totalrowsbreeders3-length(allele11q_b1_mat4(:,1)) ;

G0totalrowsbreeders3=(rows34+rows35+rows36+rows37+rows38+rows39+rows40+row
s41+rows42+rows43+rows44);

count_allele1q_b1_mat4=sum(allele1q_b1_mat4) ;
count_allele2q_b1_mat4=sum(allele2q_b1_mat4) ;
count_allele3q_b1_mat4=sum(allele3q_b1_mat4) ;
count_allele4q_b1_mat4=sum(allele4q_b1_mat4) ;
count_allele5q_b1_mat4=sum(allele5q_b1_mat4) ;
count_allele6q_b1_mat4=sum(allele6q_b1_mat4) ;
count_allele7q_b1_mat4=sum(allele7q_b1_mat4) ;
count_allele8q_b1_mat4=sum(allele8q_b1_mat4) ;
count_allele9q_b1_mat4=sum(allele9q_b1_mat4) ;
count_allele10q_b1_mat4=sum(allele10q_b1_mat4) ;
count_allele11q_b1_mat4=sum(allele11q_b1_mat4) ;

freqallele1q_b1_mat4=sum(count_allele1q_b1_mat4)/(nobreeders1*2) ;
freqallele2q_b1_mat4=sum(count_allele2q_b1_mat4)/(nobreeders1*2) ;
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```
freqallele3q_b1_mat4=sum(count_allele3q_b1_mat4)/(nobreeders1*2) ;
freqallele4q_b1_mat4=sum(count_allele4q_b1_mat4)/(nobreeders1*2) ;
freqallele5q_b1_mat4=sum(count_allele5q_b1_mat4)/(nobreeders1*2) ;
freqallele6q_b1_mat4=sum(count_allele6q_b1_mat4)/(nobreeders1*2) ;
freqallele7q_b1_mat4=sum(count_allele7q_b1_mat4)/(nobreeders1*2) ;
freqallele8q_b1_mat4=sum(count_allele8q_b1_mat4)/(nobreeders1*2) ;
freqallele9q_b1_mat4=sum(count_allele9q_b1_mat4)/(nobreeders1*2) ;
freqallele10q_b1_mat4=sum(count_allele10q_b1_mat4)/(nobreeders1*2) ;
freqallele11q_b1_mat4=sum(count_allele11q_b1_mat4)/(nobreeders1*2) ;

freqallele1q_b1=freqallele1q_b1_mat1+freqallele1q_b1_mat2+freqallele1q_b1_
mat3+freqallele1q_b1_mat4;
freqallele2q_b1=freqallele2q_b1_mat1+freqallele2q_b1_mat2+freqallele2q_b1_
mat3+freqallele2q_b1_mat4;
freqallele3q_b1=freqallele3q_b1_mat1+freqallele3q_b1_mat2+freqallele3q_b1_
mat3+freqallele3q_b1_mat4;
freqallele4q_b1=freqallele4q_b1_mat1+freqallele4q_b1_mat2+freqallele4q_b1_
mat3+freqallele4q_b1_mat4;
freqallele5q_b1=freqallele5q_b1_mat1+freqallele5q_b1_mat2+freqallele5q_b1_
mat3+freqallele5q_b1_mat4;
freqallele6q_b1=freqallele6q_b1_mat1+freqallele6q_b1_mat2+freqallele6q_b1_
mat3+freqallele6q_b1_mat4;
freqallele7q_b1=freqallele7q_b1_mat1+freqallele7q_b1_mat2+freqallele7q_b1_
mat3+freqallele7q_b1_mat4;
freqallele8q_b1=freqallele8q_b1_mat1+freqallele8q_b1_mat2+freqallele8q_b1_
mat3+freqallele8q_b1_mat4;
freqallele9q_b1=freqallele9q_b1_mat1+freqallele9q_b1_mat2+freqallele9q_b1_
mat3+freqallele9q_b1_mat4;
freqallele10q_b1=freqallele10q_b1_mat1+freqallele10q_b1_mat2+freqallele10q
_b1_mat3+freqallele10q_b1_mat4;
freqallele11q_b1=freqallele11q_b1_mat1+freqallele11q_b1_mat2+freqallele11q
_b1_mat3+freqallele11q_b1_mat4;
%These (this) queen genotype(s) had mated with (n) drones in last
generation
%Hence breeder contribution is

Ballelefreq1_G1=(2*freqallele1q_b1+freqallele1d_G0)/3
Ballelefreq2_G1=(2*freqallele2q_b1+freqallele2d_G0)/3
Ballelefreq3_G1=(2*freqallele3q_b1+freqallele3d_G0)/3
Ballelefreq4_G1=(2*freqallele4q_b1+freqallele4d_G0)/3
Ballelefreq5_G1=(2*freqallele5q_b1+freqallele5d_G0)/3
Ballelefreq6_G1=(2*freqallele6q_b1+freqallele6d_G0)/3
Ballelefreq7_G1=(2*freqallele7q_b1+freqallele7d_G0)/3
Ballelefreq8_G1=(2*freqallele8q_b1+freqallele8d_G0)/3
Ballelefreq9_G1=(2*freqallele9q_b1+freqallele9d_G0)/3
Ballelefreq10_G1=(2*freqallele10q_b1+freqallele10d_G0)/3
Ballelefreq11_G1=(2*freqallele11q_b1+freqallele11d_G0)/3



%GENERATE 100 QUEENS WITH ABOVE DISTRIBUTION
DistGen1Q=rand(nqueens,2) ;

allele1q_G1_mat1=(DistGen1Q>0) & (DistGen1Q<Ballelefreq1_G1);
```

```
allele2q_G1_mat1=(DistGen1Q>=Ballelefreq1_G1) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1));
allele3q_G1_mat1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1));
allele4q_G1_mat1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3
_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1));
allele5q_G1_mat1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3
_G1+Ballelefreq4_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1));
allele6q_G1_mat1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3
_G1+Ballelefreq4_G1+Ballelefreq5_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1));
allele7q_G1_mat1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3
_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1));
allele8q_G1_mat1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3
_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1));
allele9q_G1_mat1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3
_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballel
efreq8_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballelef
req9_G1));
allele10q_G1_mat1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq
3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Balle
lefreq8_G1+Ballelefreq9_G1)) &
(DistGen1Q<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4_G
1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballelef
req9_G1+Ballelefreq10_G1));
allele11q_G1_mat1=(DistGen1Q>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq
3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Balle
lefreq8_G1+Ballelefreq9_G1+Ballelefreq10_G1)) & (DistGen1Q<1);

allele1q_G1_mat1(all(allele1q_G1_mat1==1,2),:)=[];
allele2q_G1_mat1(all(allele2q_G1_mat1==1,2),:)=[];
allele3q_G1_mat1(all(allele3q_G1_mat1==1,2),:)=[];
allele4q_G1_mat1(all(allele4q_G1_mat1==1,2),:)=[];
allele5q_G1_mat1(all(allele5q_G1_mat1==1,2),:)=[];
allele6q_G1_mat1(all(allele6q_G1_mat1==1,2),:)=[];
allele7q_G1_mat1(all(allele7q_G1_mat1==1,2),:)=[];
allele8q_G1_mat1(all(allele8q_G1_mat1==1,2),:)=[];
allele9q_G1_mat1(all(allele9q_G1_mat1==1,2),:)=[];
allele10q_G1_mat1(all(allele10q_G1_mat1==1,2),:)=[];
allele11q_G1_mat1(all(allele11q_G1_mat1==1,2),:)=[];

rows1=nqueens-length(allele1q_G1_mat1(:,1));
rows2=nqueens-length(allele2q_G1_mat1(:,1));
rows3=nqueens-length(allele3q_G1_mat1(:,1));
```

```
rows4=nqueens-length(allele4q_G1_mat1(:,1));
rows5=nqueens-length(allele5q_G1_mat1(:,1));
rows6=nqueens-length(allele6q_G1_mat1(:,1));
rows7=nqueens-length(allele7q_G1_mat1(:,1));
rows8=nqueens-length(allele8q_G1_mat1(:,1));
rows9=nqueens-length(allele9q_G1_mat1(:,1));
rows10=nqueens-length(allele10q_G1_mat1(:,1));
rows11=nqueens-length(allele11q_G1_mat1(:,1));

totalrows1=(rows1+rows2+rows3+rows4+rows5+rows6+rows7+rows8+rows9+rows10+r
ows11);

count_allele1q_G1_mat1=sum(allele1q_G1_mat1) ;
count_allele2q_G1_mat1=sum(allele2q_G1_mat1) ;
count_allele3q_G1_mat1=sum(allele3q_G1_mat1) ;
count_allele4q_G1_mat1=sum(allele4q_G1_mat1) ;
count_allele5q_G1_mat1=sum(allele5q_G1_mat1) ;
count_allele6q_G1_mat1=sum(allele6q_G1_mat1) ;
count_allele7q_G1_mat1=sum(allele7q_G1_mat1) ;
count_allele8q_G1_mat1=sum(allele8q_G1_mat1) ;
count_allele9q_G1_mat1=sum(allele9q_G1_mat1) ;
count_allele10q_G1_mat1=sum(allele10q_G1_mat1) ;
count_allele11q_G1_mat1=sum(allele11q_G1_mat1) ;

freqallele1q_G1_mat1=sum(count_allele1q_G1_mat1)/(nqueens*2);
freqallele2q_G1_mat1=sum(count_allele2q_G1_mat1)/(nqueens*2);
freqallele3q_G1_mat1=sum(count_allele3q_G1_mat1)/(nqueens*2);
freqallele4q_G1_mat1=sum(count_allele4q_G1_mat1)/(nqueens*2);
freqallele5q_G1_mat1=sum(count_allele5q_G1_mat1)/(nqueens*2);
freqallele6q_G1_mat1=sum(count_allele6q_G1_mat1)/(nqueens*2);
freqallele7q_G1_mat1=sum(count_allele7q_G1_mat1)/(nqueens*2);
freqallele8q_G1_mat1=sum(count_allele8q_G1_mat1)/(nqueens*2);
freqallele9q_G1_mat1=sum(count_allele9q_G1_mat1)/(nqueens*2);
freqallele10q_G1_mat1=sum(count_allele10q_G1_mat1)/(nqueens*2);
freqallele11q_G1_mat1=sum(count_allele11q_G1_mat1)/(nqueens*2);

DistGen1Q_2=rand(totalrows1,2) ;

allele1q_G1_mat2=(DistGen1Q_2>0) & (DistGen1Q_2<Ballelefreq1_G1);
allele2q_G1_mat2=(DistGen1Q_2>=Ballelefreq1_G1) &
(DistGen1Q_2<(Ballelefreq1_G1+Ballelefreq2_G1));
allele3q_G1_mat2=(DistGen1Q_2>=(Ballelefreq1_G1+Ballelefreq2_G1)) &
(DistGen1Q_2<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1));
allele4q_G1_mat2=(DistGen1Q_2>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1)) &
(DistGen1Q_2<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1));
allele5q_G1_mat2=(DistGen1Q_2>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1)) &
(DistGen1Q_2<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1));
allele6q_G1_mat2=(DistGen1Q_2>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1)) &
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(DistGen1Q_2<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1));
allele7q_G1_mat2=(DistGen1Q_2>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1)) &
(DistGen1Q_2<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1));
allele8q_G1_mat2=(DistGen1Q_2>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1)) &
(DistGen1Q_2<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1));
allele9q_G1_mat2=(DistGen1Q_2>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ball
elefreq8_G1)) &
(DistGen1Q_2<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballel
efreq9_G1));
allele10q_G1_mat2=(DistGen1Q_2>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefr
eq3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Bal
lelefreq8_G1+Ballelefreq9_G1)) &
(DistGen1Q_2<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballel
efreq9_G1+Ballelefreq10_G1));
allele11q_G1_mat2=(DistGen1Q_2>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefr
eq3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Bal
lelefreq8_G1+Ballelefreq9_G1+Ballelefreq10_G1)) & (DistGen1Q_2<1);

allele1q_G1_mat2(all(allele1q_G1_mat2==1,2),:)=[] ;
allele2q_G1_mat2(all(allele2q_G1_mat2==1,2),:)=[] ;
allele3q_G1_mat2(all(allele3q_G1_mat2==1,2),:)=[] ;
allele4q_G1_mat2(all(allele4q_G1_mat2==1,2),:)=[] ;
allele5q_G1_mat2(all(allele5q_G1_mat2==1,2),:)=[] ;
allele6q_G1_mat2(all(allele6q_G1_mat2==1,2),:)=[] ;
allele7q_G1_mat2(all(allele7q_G1_mat2==1,2),:)=[] ;
allele8q_G1_mat2(all(allele8q_G1_mat2==1,2),:)=[] ;
allele9q_G1_mat2(all(allele9q_G1_mat2==1,2),:)=[] ;
allele10q_G1_mat2(all(allele10q_G1_mat2==1,2),:)=[] ;
allele11q_G1_mat2(all(allele11q_G1_mat2==1,2),:)=[] ;

rows12=totalrows1-length(allele1q_G1_mat2(:,1));
rows13=totalrows1-length(allele2q_G1_mat2(:,1));
rows14=totalrows1-length(allele3q_G1_mat2(:,1));
rows15=totalrows1-length(allele4q_G1_mat2(:,1));
rows16=totalrows1-length(allele5q_G1_mat2(:,1));
rows17=totalrows1-length(allele6q_G1_mat2(:,1));
rows18=totalrows1-length(allele7q_G1_mat2(:,1));
rows19=totalrows1-length(allele8q_G1_mat2(:,1));
rows20=totalrows1-length(allele9q_G1_mat2(:,1));
rows21=totalrows1-length(allele10q_G1_mat2(:,1));
rows22=totalrows1-length(allele11q_G1_mat2(:,1));

totalrows2=(rows12+rows13+rows14+rows15+rows16+rows17+rows18+rows19+rows20
+rows21+rows22);

count_allele1q_G1_mat2=sum(allele1q_G1_mat2) ;
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count_allele2q_G1_mat2=sum(allele2q_G1_mat2) ;
count_allele3q_G1_mat2=sum(allele3q_G1_mat2) ;
count_allele4q_G1_mat2=sum(allele4q_G1_mat2) ;
count_allele5q_G1_mat2=sum(allele5q_G1_mat2) ;
count_allele6q_G1_mat2=sum(allele6q_G1_mat2) ;
count_allele7q_G1_mat2=sum(allele7q_G1_mat2) ;
count_allele8q_G1_mat2=sum(allele8q_G1_mat2) ;
count_allele9q_G1_mat2=sum(allele9q_G1_mat2) ;
count_allele10q_G1_mat2=sum(allele10q_G1_mat2) ;
count_allele11q_G1_mat2=sum(allele11q_G1_mat2) ;

freqallele1q_G1_mat2=sum(count_allele1q_G1_mat2)/(nqueens*2);
freqallele2q_G1_mat2=sum(count_allele2q_G1_mat2)/(nqueens*2);
freqallele3q_G1_mat2=sum(count_allele3q_G1_mat2)/(nqueens*2);
freqallele4q_G1_mat2=sum(count_allele4q_G1_mat2)/(nqueens*2);
freqallele5q_G1_mat2=sum(count_allele5q_G1_mat2)/(nqueens*2);
freqallele6q_G1_mat2=sum(count_allele6q_G1_mat2)/(nqueens*2);
freqallele7q_G1_mat2=sum(count_allele7q_G1_mat2)/(nqueens*2);
freqallele8q_G1_mat2=sum(count_allele8q_G1_mat2)/(nqueens*2);
freqallele9q_G1_mat2=sum(count_allele9q_G1_mat2)/(nqueens*2);
freqallele10q_G1_mat2=sum(count_allele10q_G1_mat2)/(nqueens*2);
freqallele11q_G1_mat2=sum(count_allele11q_G1_mat2)/(nqueens*2);

DistGen1Q_3=rand(totalrows2,2);

allele1q_G1_mat3=(DistGen1Q_3>0) & (DistGen1Q_3<Ballelefreq1_G1);
allele2q_G1_mat3=(DistGen1Q_3>=Ballelefreq1_G1) &
(DistGen1Q_3<(Ballelefreq1_G1+Ballelefreq2_G1));
allele3q_G1_mat3=(DistGen1Q_3>=(Ballelefreq1_G1+Ballelefreq2_G1)) &
(DistGen1Q_3<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1));
allele4q_G1_mat3=(DistGen1Q_3>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1)) &
(DistGen1Q_3<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1));
allele5q_G1_mat3=(DistGen1Q_3>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1)) &
(DistGen1Q_3<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1));
allele6q_G1_mat3=(DistGen1Q_3>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1)) &
(DistGen1Q_3<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1));
allele7q_G1_mat3=(DistGen1Q_3>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1)) &
(DistGen1Q_3<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1));
allele8q_G1_mat3=(DistGen1Q_3>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1)) &
(DistGen1Q_3<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1));
allele9q_G1_mat3=(DistGen1Q_3>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ball
elefreq8_G1)) &
(DistGen1Q_3<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
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_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballel
efreq9_G1));
allele10q_G1_mat3=(DistGen1Q_3>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefr
eq3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Bal
lelefreq8_G1+Ballelefreq9_G1)) &
(DistGen1Q_3<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballel
efreq9_G1+Ballelefreq10_G1));
allele11q_G1_mat3=(DistGen1Q_3>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefr
eq3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Bal
lelefreq8_G1+Ballelefreq9_G1+Ballelefreq10_G1)) & (DistGen1Q_3<1);

allele1q_G1_mat3(all(allele1q_G1_mat3==1,2),:)=[];
allele2q_G1_mat3(all(allele2q_G1_mat3==1,2),:)=[];
allele3q_G1_mat3(all(allele3q_G1_mat3==1,2),:)=[];
allele4q_G1_mat3(all(allele4q_G1_mat3==1,2),:)=[];
allele5q_G1_mat3(all(allele5q_G1_mat3==1,2),:)=[];
allele6q_G1_mat3(all(allele6q_G1_mat3==1,2),:)=[];
allele7q_G1_mat3(all(allele7q_G1_mat3==1,2),:)=[];
allele8q_G1_mat3(all(allele8q_G1_mat3==1,2),:)=[];
allele9q_G1_mat3(all(allele9q_G1_mat3==1,2),:)=[];
allele10q_G1_mat3(all(allele10q_G1_mat3==1,2),:)=[];
allele11q_G1_mat3(all(allele11q_G1_mat3==1,2),:)=[];

rows23=totalrows2-length(allele1q_G1_mat3(:,1)) ;
rows24=totalrows2-length(allele2q_G1_mat3(:,1)) ;
rows25=totalrows2-length(allele3q_G1_mat3(:,1)) ;
rows26=totalrows2-length(allele4q_G1_mat3(:,1)) ;
rows27=totalrows2-length(allele5q_G1_mat3(:,1)) ;
rows28=totalrows2-length(allele6q_G1_mat3(:,1)) ;
rows29=totalrows2-length(allele7q_G1_mat3(:,1)) ;
rows30=totalrows2-length(allele8q_G1_mat3(:,1)) ;
rows31=totalrows2-length(allele9q_G1_mat3(:,1)) ;
rows32=totalrows2-length(allele10q_G1_mat3(:,1)) ;
rows33=totalrows2-length(allele11q_G1_mat3(:,1)) ;

totalrows3=(rows23+rows24+rows25+rows26+rows27+rows28+rows29+rows30+rows31
+rows32+rows33);

count_allele1q_G1_mat3=sum(allele1q_G1_mat3) ;
count_allele2q_G1_mat3=sum(allele2q_G1_mat3) ;
count_allele3q_G1_mat3=sum(allele3q_G1_mat3) ;
count_allele4q_G1_mat3=sum(allele4q_G1_mat3) ;
count_allele5q_G1_mat3=sum(allele5q_G1_mat3);
count_allele6q_G1_mat3=sum(allele6q_G1_mat3) ;
count_allele7q_G1_mat3=sum(allele7q_G1_mat3) ;
count_allele8q_G1_mat3=sum(allele8q_G1_mat3) ;
count_allele9q_G1_mat3=sum(allele9q_G1_mat3) ;
count_allele10q_G1_mat3=sum(allele10q_G1_mat3) ;
count_allele11q_G1_mat3=sum(allele11q_G1_mat3) ;

freqallele1q_G1_mat3=sum(count_allele1q_G1_mat3)/(nqueens*2) ;
freqallele2q_G1_mat3=sum(count_allele2q_G1_mat3)/(nqueens*2) ;
freqallele3q_G1_mat3=sum(count_allele3q_G1_mat3)/(nqueens*2) ;
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freqallele4q_G1_mat3=sum(count_allele4q_G1_mat3)/(nqueens*2) ;
freqallele5q_G1_mat3=sum(count_allele5q_G1_mat3)/(nqueens*2) ;
freqallele6q_G1_mat3=sum(count_allele6q_G1_mat3)/(nqueens*2) ;
freqallele7q_G1_mat3=sum(count_allele7q_G1_mat3)/(nqueens*2) ;
freqallele8q_G1_mat3=sum(count_allele8q_G1_mat3)/(nqueens*2) ;
freqallele9q_G1_mat3=sum(count_allele9q_G1_mat3)/(nqueens*2) ;
freqallele10q_G1_mat3=sum(count_allele10q_G1_mat3)/(nqueens*2) ;
freqallele11q_G1_mat3=sum(count_allele11q_G1_mat3)/(nqueens*2) ;

DistGen1Q_4=rand(totalrows3,2);

allele1q_G1_mat4=(DistGen1Q_4>0) & (DistGen1Q_4<Ballelefreq1_G1);
allele2q_G1_mat4=(DistGen1Q_4>=Ballelefreq1_G1) &
(DistGen1Q_4<(Ballelefreq1_G1+Ballelefreq2_G1));
allele3q_G1_mat4=(DistGen1Q_4>=(Ballelefreq1_G1+Ballelefreq2_G1)) &
(DistGen1Q_4<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1));
allele4q_G1_mat4=(DistGen1Q_4>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1)) &
(DistGen1Q_4<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1));
allele5q_G1_mat4=(DistGen1Q_4>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1)) &
(DistGen1Q_4<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1));
allele6q_G1_mat4=(DistGen1Q_4>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1)) &
(DistGen1Q_4<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1));
allele7q_G1_mat4=(DistGen1Q_4>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1)) &
(DistGen1Q_4<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1));
allele8q_G1_mat4=(DistGen1Q_4>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1)) &
(DistGen1Q_4<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1));
allele9q_G1_mat4=(DistGen1Q_4>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ball
elefreq8_G1)) &
(DistGen1Q_4<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballel
efreq9_G1));
allele10q_G1_mat4=(DistGen1Q_4>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefr
eq3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Bal
lelefreq8_G1+Ballelefreq9_G1)) &
(DistGen1Q_4<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballel
efreq9_G1+Ballelefreq10_G1));
allele11q_G1_mat4=(DistGen1Q_4>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefr
eq3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Bal
lelefreq8_G1+Ballelefreq9_G1+Ballelefreq10_G1)) & (DistGen1Q_4<1);


allele1q_G1_mat4(all(allele1q_G1_mat4==1,2),:)=[] ;
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allele2q_G1_mat4(all(allele2q_G1_mat4==1,2),:)=[] ;
allele3q_G1_mat4(all(allele3q_G1_mat4==1,2),:)=[] ;
allele4q_G1_mat4(all(allele4q_G1_mat4==1,2),:)=[] ;
allele5q_G1_mat4(all(allele5q_G1_mat4==1,2),:)=[] ;
allele6q_G1_mat4(all(allele6q_G1_mat4==1,2),:)=[] ;
allele7q_G1_mat4(all(allele7q_G1_mat4==1,2),:)=[] ;
allele8q_G1_mat4(all(allele8q_G1_mat4==1,2),:)=[] ;
allele9q_G1_mat4(all(allele9q_G1_mat4==1,2),:)=[] ;
allele10q_G1_mat4(all(allele10q_G1_mat4==1,2),:)=[] ;
allele11q_G1_mat4(all(allele11q_G1_mat4==1,2),:)=[] ;

rows34=totalrows3-length(allele1q_G1_mat4(:,1)) ;
rows35=totalrows3-length(allele2q_G1_mat4(:,1)) ;
rows36=totalrows3-length(allele3q_G1_mat4(:,1)) ;
rows37=totalrows3-length(allele4q_G1_mat4(:,1)) ;
rows38=totalrows3-length(allele5q_G1_mat4(:,1)) ;
rows39=totalrows3-length(allele6q_G1_mat4(:,1)) ;
rows40=totalrows3-length(allele7q_G1_mat4(:,1)) ;
rows41=totalrows3-length(allele8q_G1_mat4(:,1)) ;
rows42=totalrows3-length(allele9q_G1_mat4(:,1)) ;
rows43=totalrows3-length(allele10q_G1_mat4(:,1)) ;
rows44=totalrows3-length(allele11q_G1_mat4(:,1));

totalrows4=(rows34+rows35+rows36+rows37+rows38+rows39+rows40+rows41+rows42
+rows43+rows44);

count_allele1q_G1_mat4=sum(allele1q_G1_mat4) ;
count_allele2q_G1_mat4=sum(allele2q_G1_mat4) ;
count_allele3q_G1_mat4=sum(allele3q_G1_mat4) ;
count_allele4q_G1_mat4=sum(allele4q_G1_mat4) ;
count_allele5q_G1_mat4=sum(allele5q_G1_mat4) ;
count_allele6q_G1_mat4=sum(allele6q_G1_mat4) ;
count_allele7q_G1_mat4=sum(allele7q_G1_mat4) ;
count_allele8q_G1_mat4=sum(allele8q_G1_mat4) ;
count_allele9q_G1_mat4=sum(allele9q_G1_mat4) ;
count_allele10q_G1_mat4=sum(allele10q_G1_mat4) ;
count_allele11q_G1_mat4=sum(allele11q_G1_mat4) ;

freqallele1q_G1_mat4=sum(count_allele1q_G1_mat4)/(nqueens*2) ;
freqallele2q_G1_mat4=sum(count_allele2q_G1_mat4)/(nqueens*2) ;
freqallele3q_G1_mat4=sum(count_allele3q_G1_mat4)/(nqueens*2) ;
freqallele4q_G1_mat4=sum(count_allele4q_G1_mat4)/(nqueens*2) ;
freqallele5q_G1_mat4=sum(count_allele5q_G1_mat4)/(nqueens*2) ;
freqallele6q_G1_mat4=sum(count_allele6q_G1_mat4)/(nqueens*2) ;
freqallele7q_G1_mat4=sum(count_allele7q_G1_mat4)/(nqueens*2) ;
freqallele8q_G1_mat4=sum(count_allele8q_G1_mat4)/(nqueens*2) ;
freqallele9q_G1_mat4=sum(count_allele9q_G1_mat4)/(nqueens*2) ;
freqallele10q_G1_mat4=sum(count_allele10q_G1_mat4)/(nqueens*2) ;
freqallele11q_G1_mat4=sum(count_allele11q_G1_mat4)/(nqueens*2) ;

DistGen1Q_5=rand(totalrows4,2);

allele1q_G1_mat5=(DistGen1Q_5>0) & (DistGen1Q_5<Ballelefreq1_G1);
```

```
allele2q_G1_mat5=(DistGen1Q_5>=Ballelefreq1_G1) &
(DistGen1Q_5<(Ballelefreq1_G1+Ballelefreq2_G1));
allele3q_G1_mat5=(DistGen1Q_5>=(Ballelefreq1_G1+Ballelefreq2_G1)) &
(DistGen1Q_5<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1));
allele4q_G1_mat5=(DistGen1Q_5>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1)) &
(DistGen1Q_5<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1));
allele5q_G1_mat5=(DistGen1Q_5>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1)) &
(DistGen1Q_5<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1));
allele6q_G1_mat5=(DistGen1Q_5>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1)) &
(DistGen1Q_5<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1));
allele7q_G1_mat5=(DistGen1Q_5>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1)) &
(DistGen1Q_5<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1));
allele8q_G1_mat5=(DistGen1Q_5>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1)) &
(DistGen1Q_5<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1));
allele9q_G1_mat5=(DistGen1Q_5>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefre
q3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ball
elefreq8_G1)) &
(DistGen1Q_5<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballel
efreq9_G1));
allele10q_G1_mat5=(DistGen1Q_5>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefr
eq3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Bal
lelefreq8_G1+Ballelefreq9_G1)) &
(DistGen1Q_5<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq4
_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Ballel
efreq9_G1+Ballelefreq10_G1));
allele11q_G1_mat5=(DistGen1Q_5>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefr
eq3_G1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Bal
lelefreq8_G1+Ballelefreq9_G1+Ballelefreq10_G1)) & (DistGen1Q_5<1);


allele1q_G1_mat5(all(allele1q_G1_mat5==1,2),:)=[];
allele2q_G1_mat5(all(allele2q_G1_mat5==1,2),:)=[];
allele3q_G1_mat5(all(allele3q_G1_mat5==1,2),:)=[];
allele4q_G1_mat5(all(allele4q_G1_mat5==1,2),:)=[];
allele5q_G1_mat5(all(allele5q_G1_mat5==1,2),:)=[];
allele6q_G1_mat5(all(allele6q_G1_mat5==1,2),:)=[];
allele7q_G1_mat5(all(allele7q_G1_mat5==1,2),:)=[];
allele8q_G1_mat5(all(allele8q_G1_mat5==1,2),:)=[];
allele9q_G1_mat5(all(allele9q_G1_mat5==1,2),:)=[];
allele10q_G1_mat5(all(allele10q_G1_mat5==1,2),:)=[];
allele11q_G1_mat5(all(allele11q_G1_mat5==1,2),:)=[];

rows45=totalrows4-length(allele1q_G1_mat5(:,1));
rows46=totalrows4-length(allele2q_G1_mat5(:,1));
```

```
rows47=totalrows4-length(allele3q_G1_mat5(:,1));
rows48=totalrows4-length(allele4q_G1_mat5(:,1));
rows49=totalrows4-length(allele5q_G1_mat5(:,1));
rows50=totalrows4-length(allele6q_G1_mat5(:,1));
rows51=totalrows4-length(allele7q_G1_mat5(:,1));
rows52=totalrows4-length(allele8q_G1_mat5(:,1));
rows53=totalrows4-length(allele9q_G1_mat5(:,1));
rows54=totalrows4-length(allele10q_G1_mat5(:,1));
rows55=totalrows4-length(allele11q_G1_mat5(:,1));

totalrows5=(rows45+rows46+rows47+rows48+rows49+rows50+rows51+rows52+rows53
+rows54+rows55);

count_allele1q_G1_mat5=sum(allele1q_G1_mat5);
count_allele2q_G1_mat5=sum(allele2q_G1_mat5);
count_allele3q_G1_mat5=sum(allele3q_G1_mat5);
count_allele4q_G1_mat5=sum(allele4q_G1_mat5);
count_allele5q_G1_mat5=sum(allele5q_G1_mat5);
count_allele6q_G1_mat5=sum(allele6q_G1_mat5);
count_allele7q_G1_mat5=sum(allele7q_G1_mat5);
count_allele8q_G1_mat5=sum(allele8q_G1_mat5);
count_allele9q_G1_mat5=sum(allele9q_G1_mat5);
count_allele10q_G1_mat5=sum(allele10q_G1_mat5);
count_allele11q_G1_mat5=sum(allele11q_G1_mat5);

freqallele1q_G1_mat5=sum(count_allele1q_G1_mat5)/(nqueens*2);
freqallele2q_G1_mat5=sum(count_allele2q_G1_mat5)/(nqueens*2);
freqallele3q_G1_mat5=sum(count_allele3q_G1_mat5)/(nqueens*2);
freqallele4q_G1_mat5=sum(count_allele4q_G1_mat5)/(nqueens*2);
freqallele5q_G1_mat5=sum(count_allele5q_G1_mat5)/(nqueens*2);
freqallele6q_G1_mat5=sum(count_allele6q_G1_mat5)/(nqueens*2);
freqallele7q_G1_mat5=sum(count_allele7q_G1_mat5)/(nqueens*2);
freqallele8q_G1_mat5=sum(count_allele8q_G1_mat5)/(nqueens*2);
freqallele9q_G1_mat5=sum(count_allele9q_G1_mat5)/(nqueens*2);
freqallele10q_G1_mat5=sum(count_allele10q_G1_mat5)/(nqueens*2);
freqallele11q_G1_mat5=sum(count_allele11q_G1_mat5)/(nqueens*2);

freqallele1q_G1=freqallele1q_G1_mat1+freqallele1q_G1_mat2+freqallele1q_G1_
mat3+freqallele1q_G1_mat4+freqallele1q_G1_mat5;
freqallele2q_G1=freqallele2q_G1_mat1+freqallele2q_G1_mat2+freqallele2q_G1_
mat3+freqallele2q_G1_mat4+freqallele2q_G1_mat5;
freqallele3q_G1=freqallele3q_G1_mat1+freqallele3q_G1_mat2+freqallele3q_G1_
mat3+freqallele3q_G1_mat4+freqallele3q_G1_mat5;
freqallele4q_G1=freqallele4q_G1_mat1+freqallele4q_G1_mat2+freqallele4q_G1_
mat3+freqallele4q_G1_mat4+freqallele4q_G1_mat5;
freqallele5q_G1=freqallele5q_G1_mat1+freqallele5q_G1_mat2+freqallele5q_G1_
mat3+freqallele5q_G1_mat4+freqallele5q_G1_mat5;
freqallele6q_G1=freqallele6q_G1_mat1+freqallele6q_G1_mat2+freqallele6q_G1_
mat3+freqallele6q_G1_mat4+freqallele6q_G1_mat5;
freqallele7q_G1=freqallele7q_G1_mat1+freqallele7q_G1_mat2+freqallele7q_G1_
mat3+freqallele7q_G1_mat4+freqallele7q_G1_mat5;
freqallele8q_G1=freqallele8q_G1_mat1+freqallele8q_G1_mat2+freqallele8q_G1_
mat3+freqallele8q_G1_mat4+freqallele8q_G1_mat5;
```

```
freqallele9q_G1=freqallele9q_G1_mat1+freqallele9q_G1_mat2+freqallele9q_G1_
mat3+freqallele9q_G1_mat4+freqallele9q_G1_mat5;
freqallele10q_G1=freqallele10q_G1_mat1+freqallele10q_G1_mat2+freqallele10q
_G1_mat3+freqallele10q_G1_mat4+freqallele10q_G1_mat5;
freqallele11q_G1=freqallele11q_G1_mat1+freqallele11q_G1_mat2+freqallele11q
_G1_mat3+freqallele11q_G1_mat4+freqallele11q_G1_mat5;

freqallele1_G1=(2*freqallele1q_G1+freqallele1d_G0)/3;
freqallele2_G1=(2*freqallele2q_G1+freqallele2d_G0)/3;
freqallele3_G1=(2*freqallele3q_G1+freqallele3d_G0)/3;
freqallele4_G1=(2*freqallele4q_G1+freqallele4d_G0)/3;
freqallele5_G1=(2*freqallele5q_G1+freqallele5d_G0)/3;
freqallele6_G1=(2*freqallele6q_G1+freqallele6d_G0)/3;
freqallele7_G1=(2*freqallele7q_G1+freqallele7d_G0)/3;
freqallele8_G1=(2*freqallele8q_G1+freqallele8d_G0)/3;
freqallele9_G1=(2*freqallele9q_G1+freqallele9d_G0)/3;
freqallele10_G1=(2*freqallele10q_G1+freqallele10d_G0)/3;
freqallele11_G1=(2*freqallele11q_G1+freqallele11d_G0)/3;



mat1_G1(freqallele1)=freqallele1_G1;
mat2_G1(freqallele1)=freqallele2_G1;
mat3_G1(freqallele1)=freqallele3_G1;
mat4_G1(freqallele1)=freqallele4_G1;
mat5_G1(freqallele1)=freqallele5_G1;
mat6_G1(freqallele1)=freqallele6_G1;
mat7_G1(freqallele1)=freqallele7_G1;
mat8_G1(freqallele1)=freqallele8_G1;
mat9_G1(freqallele1)=freqallele9_G1;
mat10_G1(freqallele1)=freqallele10_G1;
mat11_G1(freqallele1)=freqallele11_G1;

%OKOKOKOK


%SECOND GENERATION SIMULTION
%ALLELE FREQUENCIES CARRIED BY DRONES CONTRIBUTING TO NEXT GENERATION
COMES
%FROM QUEENS SELECTED AS BREEDERS LAST YEAR ie Ballelefreqx_G1

DistG1drones=rand(nqueens,nodrones)


allele1d_1=(DistG1drones>0) & (DistG1drones<Ballelefreq1_G1);
allele2d_1=(DistG1drones>=Ballelefreq1_G1) &
(DistG1drones<(Ballelefreq1_G1+Ballelefreq2_G1));
allele3d_1=(DistG1drones>=(Ballelefreq1_G1+Ballelefreq2_G1)) &
(DistG1drones<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1));
allele4d_1=(DistG1drones>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1
)) &
(DistG1drones<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq
4_G1));
```

```
allele5d_1=(DistG1drones>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1
+Ballelefreq4_G1)) &
(DistG1drones<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq
4_G1+Ballelefreq5_G1));
allele6d_1=(DistG1drones>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1
+Ballelefreq4_G1+Ballelefreq5_G1)) &
(DistG1drones<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq
4_G1+Ballelefreq5_G1+Ballelefreq6_G1));
allele7d_1=(DistG1drones>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1
+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1)) &
(DistG1drones<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq
4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1));
allele8d_1=(DistG1drones>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1
+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1)) &
(DistG1drones<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq
4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1));
allele9d_1=(DistG1drones>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1
+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefr
eq8_G1)) &
(DistG1drones<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq
4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Balle
lefreq9_G1));
allele10d_1=(DistG1drones>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G
1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelef
req8_G1+Ballelefreq9_G1)) &
(DistG1drones<(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G1+Ballelefreq
4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelefreq8_G1+Balle
lefreq9_G1+Ballelefreq10_G1));
allele11d_1=(DistG1drones>=(Ballelefreq1_G1+Ballelefreq2_G1+Ballelefreq3_G
1+Ballelefreq4_G1+Ballelefreq5_G1+Ballelefreq6_G1+Ballelefreq7_G1+Ballelef
req8_G1+Ballelefreq9_G1+Ballelefreq10_G1)) & (DistG1drones<1);

count_allele1d_1=sum(allele1d_1)
count_allele2d_1=sum(allele2d_1)
count_allele3d_1=sum(allele3d_1)
count_allele4d_1=sum(allele4d_1)
count_allele5d_1=sum(allele5d_1)
count_allele6d_1=sum(allele6d_1)
count_allele7d_1=sum(allele7d_1)
count_allele8d_1=sum(allele8d_1)
count_allele9d_1=sum(allele9d_1)
count_allele10d_1=sum(allele10d_1)
count_allele11d_1=sum(allele11d_1)

freqallele1d_G1=sum(count_allele1d_1)/(nqueens*nodrones)
freqallele2d_G1=sum(count_allele2d_1)/(nqueens*nodrones)
freqallele3d_G1=sum(count_allele3d_1)/(nqueens*nodrones)
freqallele4d_G1=sum(count_allele4d_1)/(nqueens*nodrones)
freqallele5d_G1=sum(count_allele5d_1)/(nqueens*nodrones)
freqallele6d_G1=sum(count_allele6d_1)/(nqueens*nodrones)
freqallele7d_G1=sum(count_allele7d_1)/(nqueens*nodrones)
freqallele8d_G1=sum(count_allele8d_1)/(nqueens*nodrones)
freqallele9d_G1=sum(count_allele9d_1)/(nqueens*nodrones)
freqallele10d_G1=sum(count_allele10d_1)/(nqueens*nodrones)
freqallele11d_G1=sum(count_allele11d_1)/(nqueens*nodrones)
```

```
%SELECT BREEDERS FROM THE G1 DISTRIBUTION
DistG1=rand(nqueens,2) ;
Breeders2=datasample(DistG1,nobreeders2) ;

allele1q_b2_mat1=(Breeders2>0) & (Breeders2<freqallele1_G1) ;
allele2q_b2_mat1=(Breeders2>=freqallele1_G1) &
(Breeders2<(freqallele1_G1+freqallele2_G1)) ;
allele3q_b2_mat1=(Breeders2>=(freqallele1_G1+freqallele2_G1)) &
(Breeders2<(freqallele1_G1+freqallele2_G1+freqallele3_G1)) ;
allele4q_b2_mat1=(Breeders2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1
)) &
(Breeders2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1))
;
allele5q_b2_mat1=(Breeders2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1
+freqallele4_G1)) &
(Breeders2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+fr
eqallele5_G1)) ;
allele6q_b2_mat1=(Breeders2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1
+freqallele4_G1+freqallele5_G1)) &
(Breeders2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+fr
eqallele5_G1+freqallele6_G1)) ;
allele7q_b2_mat1=(Breeders2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1
+freqallele4_G1+freqallele5_G1+freqallele6_G1)) &
(Breeders2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+fr
eqallele5_G1+freqallele6_G1+freqallele7_G1)) ;
allele8q_b2_mat1=(Breeders2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1
+freqallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1)) &
(Breeders2<freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+fre
qallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1) ;
allele9q_b2_mat1=(Breeders2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1
+freqallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G
1)) &
(Breeders2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+fr
eqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+freqallele9_G1))
;
allele10q_b2_mat1=(Breeders2>=(freqallele1_G1+freqallele2_G1+freqallele3_G
1+freqallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_
G1+freqallele9_G1)) &
(Breeders2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+fr
eqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+freqallele9_G1+f
reqallele10_G1)) ;
allele11q_b2_mat1=(Breeders2>=(freqallele1_G1+freqallele2_G1+freqallele3_G
1+freqallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_
G1+freqallele9_G1+freqallele10_G1)) & (Breeders2<1) ;

allele1q_b2_mat1(all(allele1q_b2_mat1==1,2),:)=[];
allele2q_b2_mat1(all(allele2q_b2_mat1==1,2),:)=[];
allele3q_b2_mat1(all(allele3q_b2_mat1==1,2),:)=[];
allele4q_b2_mat1(all(allele4q_b2_mat1==1,2),:)=[];
allele5q_b2_mat1(all(allele5q_b2_mat1==1,2),:)=[];
allele6q_b2_mat1(all(allele6q_b2_mat1==1,2),:)=[];
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allele7q_b2_mat1(all(allele7q_b2_mat1==1,2),:)=[];
allele8q_b2_mat1(all(allele8q_b2_mat1==1,2),:)=[];
allele9q_b2_mat1(all(allele9q_b2_mat1==1,2),:)=[];
allele10q_b2_mat1(all(allele10q_b2_mat1==1,2),:)=[];
allele11q_b2_mat1(all(allele11q_b2_mat1==1,2),:)=[];

rows1=nobreeders2-length(allele1q_b2_mat1(:,1));
rows2=nobreeders2-length(allele2q_b2_mat1(:,1));
rows3=nobreeders2-length(allele3q_b2_mat1(:,1));
rows4=nobreeders2-length(allele4q_b2_mat1(:,1));
rows5=nobreeders2-length(allele5q_b2_mat1(:,1));
rows6=nobreeders2-length(allele6q_b2_mat1(:,1));
rows7=nobreeders2-length(allele7q_b2_mat1(:,1));
rows8=nobreeders2-length(allele8q_b2_mat1(:,1));
rows9=nobreeders2-length(allele9q_b2_mat1(:,1));
rows10=nobreeders2-length(allele10q_b2_mat1(:,1));
rows11=nobreeders2-length(allele11q_b2_mat1(:,1));

totalrowsb1=(rows1+rows2+rows3+rows4+rows5+rows6+rows7+rows8+rows9+rows10+
rows11);

count_allele1q_b2_mat1=sum(allele1q_b2_mat1);
count_allele2q_b2_mat1=sum(allele2q_b2_mat1);
count_allele3q_b2_mat1=sum(allele3q_b2_mat1);
count_allele4q_b2_mat1=sum(allele4q_b2_mat1);
count_allele5q_b2_mat1=sum(allele5q_b2_mat1);
count_allele6q_b2_mat1=sum(allele6q_b2_mat1);
count_allele7q_b2_mat1=sum(allele7q_b2_mat1);
count_allele8q_b2_mat1=sum(allele8q_b2_mat1);
count_allele9q_b2_mat1=sum(allele9q_b2_mat1);
count_allele10q_b2_mat1=sum(allele10q_b2_mat1);
count_allele11q_b2_mat1=sum(allele11q_b2_mat1);

freqallele1q_b2_mat1=sum(count_allele1q_b2_mat1)/(nobreeders2*2);
freqallele2q_b2_mat1=sum(count_allele2q_b2_mat1)/(nobreeders2*2);
freqallele3q_b2_mat1=sum(count_allele3q_b2_mat1)/(nobreeders2*2);
freqallele4q_b2_mat1=sum(count_allele4q_b2_mat1)/(nobreeders2*2);
freqallele5q_b2_mat1=sum(count_allele5q_b2_mat1)/(nobreeders2*2);
freqallele6q_b2_mat1=sum(count_allele6q_b2_mat1)/(nobreeders2*2);
freqallele7q_b2_mat1=sum(count_allele7q_b2_mat1)/(nobreeders2*2);
freqallele8q_b2_mat1=sum(count_allele8q_b2_mat1)/(nobreeders2*2);
freqallele9q_b2_mat1=sum(count_allele9q_b2_mat1)/(nobreeders2*2);
freqallele10q_b2_mat1=sum(count_allele10q_b2_mat1)/(nobreeders2*2);
freqallele11q_b2_mat1=sum(count_allele11q_b2_mat1)/(nobreeders2*2);

dist1=rand(totalrowsb1,2) ;

allele1q_b2_mat2=(dist1>0) & (dist1<freqallele1_G1) ;
allele2q_b2_mat2=(dist1>=freqallele1_G1) &
(dist1<(freqallele1_G1+freqallele2_G1)) ;
allele3q_b2_mat2=(dist1>=(freqallele1_G1+freqallele2_G1)) &
(dist1<(freqallele1_G1+freqallele2_G1+freqallele3_G1)) ;
allele4q_b2_mat2=(dist1>=(freqallele1_G1+freqallele2_G1+freqallele3_G1)) &
(dist1<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1)) ;
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allele5q_b2_mat2=(dist1>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1)) &
(dist1<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1)) ;
allele6q_b2_mat2=(dist1>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1)) &
(dist1<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1)) ;
allele7q_b2_mat2=(dist1>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1+freqallele6_G1)) &
(dist1<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1)) ;
allele8q_b2_mat2=(dist1>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1)) &
(dist1<freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqall
ele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1) ;
allele9q_b2_mat2=(dist1>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1))
&
(dist1<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+freqallele9_G1)) ;
allele10q_b2_mat2=(dist1>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fr
eqallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+f
reqallele9_G1)) &
(dist1<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+freqallele9_G1+freqa
llele10_G1)) ;
allele11q_b2_mat2=(dist1>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fr
eqallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+f
reqallele9_G1+freqallele10_G1)) & (dist1<1) ;

allele1q_b2_mat2(all(allele1q_b2_mat2==1,2),:)=[] ;
allele2q_b2_mat2(all(allele2q_b2_mat2==1,2),:)=[] ;
allele3q_b2_mat2(all(allele3q_b2_mat2==1,2),:)=[] ;
allele4q_b2_mat2(all(allele4q_b2_mat2==1,2),:)=[] ;
allele5q_b2_mat2(all(allele5q_b2_mat2==1,2),:)=[] ;
allele6q_b2_mat2(all(allele6q_b2_mat2==1,2),:)=[] ;
allele7q_b2_mat2(all(allele7q_b2_mat2==1,2),:)=[] ;
allele8q_b2_mat2(all(allele8q_b2_mat2==1,2),:)=[] ;
allele9q_b2_mat2(all(allele9q_b2_mat2==1,2),:)=[] ;
allele10q_b2_mat2(all(allele10q_b2_mat2==1,2),:)=[] ;
allele11q_b2_mat2(all(allele11q_b2_mat2==1,2),:)=[] ;

rows12=totalrowsb1-length(allele1q_b2_mat2(:,1)) ;
rows13=totalrowsb1-length(allele2q_b2_mat2(:,1)) ;
rows14=totalrowsb1-length(allele3q_b2_mat2(:,1));
rows15=totalrowsb1-length(allele4q_b2_mat2(:,1));
rows16=totalrowsb1-length(allele5q_b2_mat2(:,1)) ;
rows17=totalrowsb1-length(allele6q_b2_mat2(:,1)) ;
rows18=totalrowsb1-length(allele7q_b2_mat2(:,1)) ;
rows19=totalrowsb1-length(allele8q_b2_mat2(:,1)) ;
rows20=totalrowsb1-length(allele9q_b2_mat2(:,1)) ;
rows21=totalrowsb1-length(allele10q_b2_mat2(:,1)) ;
rows22=totalrowsb1-length(allele11q_b2_mat2(:,1)) ;
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totalrowsb2=(rows12+rows13+rows14+rows15+rows16+rows17+rows18+rows19+rows2
0+rows21+rows22);

count_allele1q_b2_mat2=sum(allele1q_b2_mat2) ;
count_allele2q_b2_mat2=sum(allele2q_b2_mat2) ;
count_allele3q_b2_mat2=sum(allele3q_b2_mat2) ;
count_allele4q_b2_mat2=sum(allele4q_b2_mat2) ;
count_allele5q_b2_mat2=sum(allele5q_b2_mat2) ;
count_allele6q_b2_mat2=sum(allele6q_b2_mat2) ;
count_allele7q_b2_mat2=sum(allele7q_b2_mat2) ;
count_allele8q_b2_mat2=sum(allele8q_b2_mat2) ;
count_allele9q_b2_mat2=sum(allele9q_b2_mat2) ;
count_allele10q_b2_mat2=sum(allele10q_b2_mat2) ;
count_allele11q_b2_mat2=sum(allele11q_b2_mat2) ;

freqallele1q_b2_mat2=sum(count_allele1q_b2_mat2)/(nobreeders2*2) ;
freqallele2q_b2_mat2=sum(count_allele2q_b2_mat2)/(nobreeders2*2) ;
freqallele3q_b2_mat2=sum(count_allele3q_b2_mat2)/(nobreeders2*2) ;
freqallele4q_b2_mat2=sum(count_allele4q_b2_mat2)/(nobreeders2*2) ;
freqallele5q_b2_mat2=sum(count_allele5q_b2_mat2)/(nobreeders2*2) ;
freqallele6q_b2_mat2=sum(count_allele6q_b2_mat2)/(nobreeders2*2) ;
freqallele7q_b2_mat2=sum(count_allele7q_b2_mat2)/(nobreeders2*2) ;
freqallele8q_b2_mat2=sum(count_allele8q_b2_mat2)/(nobreeders2*2) ;
freqallele9q_b2_mat2=sum(count_allele9q_b2_mat2)/(nobreeders2*2) ;
freqallele10q_b2_mat2=sum(count_allele10q_b2_mat2)/(nobreeders2*2) ;
freqallele11q_b2_mat2=sum(count_allele11q_b2_mat2)/(nobreeders2*2) ;

dist2=rand(totalrowsb2,2) ;

allele1q_b2_mat3=(dist2>0) & (dist2<freqallele1_G1) ;
allele2q_b2_mat3=(dist2>=freqallele1_G1) &
(dist2<(freqallele1_G1+freqallele2_G1)) ;
allele3q_b2_mat3=(dist2>=(freqallele1_G1+freqallele2_G1)) &
(dist2<(freqallele1_G1+freqallele2_G1+freqallele3_G1)) ;
allele4q_b2_mat3=(dist2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1)) &
(dist2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1));
allele5q_b2_mat3=(dist2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1)) &
(dist2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1)) ;
allele6q_b2_mat3=(dist2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1)) &
(dist2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1)) ;
allele7q_b2_mat3=(dist2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1+freqallele6_G1)) &
(dist2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1));
allele8q_b2_mat3=(dist2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1)) &
(dist2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1)) ;
allele9q_b2_mat3=(dist2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1))
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&
(dist2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+freqallele9_G1)) ;
allele10q_b2_mat3=(dist2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fr
eqallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+f
reqallele9_G1)) &
(dist2<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+freqallele9_G1+freqa
llele10_G1));
allele11q_b2_mat3=(dist2>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fr
eqallele4_G1+freqallele5_G1+freqallele6_G1++freqallele7_G1+freqallele8_G1+
freqallele9_G1+freqallele10_G1)) & (dist2<1) ;

allele1q_b2_mat3(all(allele1q_b2_mat3==1,2),:)=[] ;
allele2q_b2_mat3(all(allele2q_b2_mat3==1,2),:)=[] ;
allele3q_b2_mat3(all(allele3q_b2_mat3==1,2),:)=[] ;
allele4q_b2_mat3(all(allele4q_b2_mat3==1,2),:)=[] ;
allele5q_b2_mat3(all(allele5q_b2_mat3==1,2),:)=[] ;
allele6q_b2_mat3(all(allele6q_b2_mat3==1,2),:)=[] ;
allele7q_b2_mat3(all(allele7q_b2_mat3==1,2),:)=[] ;
allele8q_b2_mat3(all(allele8q_b2_mat3==1,2),:)=[] ;
allele9q_b2_mat3(all(allele9q_b2_mat3==1,2),:)=[] ;
allele10q_b2_mat3(all(allele10q_b2_mat3==1,2),:)=[] ;
allele11q_b2_mat3(all(allele11q_b2_mat3==1,2),:)=[] ;

rows23=totalrowsb2-length(allele1q_b2_mat3(:,1)) ;
rows24=totalrowsb2-length(allele2q_b2_mat3(:,1));
rows25=totalrowsb2-length(allele3q_b2_mat3(:,1));
rows26=totalrowsb2-length(allele4q_b2_mat3(:,1));
rows27=totalrowsb2-length(allele5q_b2_mat3(:,1));
rows28=totalrowsb2-length(allele6q_b2_mat3(:,1));
rows29=totalrowsb2-length(allele7q_b2_mat3(:,1));
rows30=totalrowsb2-length(allele8q_b2_mat3(:,1));
rows31=totalrowsb2-length(allele9q_b2_mat3(:,1));
rows32=totalrowsb2-length(allele10q_b2_mat3(:,1));
rows33=totalrowsb2-length(allele11q_b2_mat3(:,1));

totalrowsb3=(rows23+rows24+rows25+rows26+rows27+rows28+rows29+rows30+rows3
1+rows32+rows33);

count_allele1q_b2_mat3=sum(allele1q_b2_mat3);
count_allele2q_b2_mat3=sum(allele2q_b2_mat3);
count_allele3q_b2_mat3=sum(allele3q_b2_mat3);
count_allele4q_b2_mat3=sum(allele4q_b2_mat3);
count_allele5q_b2_mat3=sum(allele5q_b2_mat3);
count_allele6q_b2_mat3=sum(allele6q_b2_mat3);
count_allele7q_b2_mat3=sum(allele7q_b2_mat3);
count_allele8q_b2_mat3=sum(allele8q_b2_mat3);
count_allele9q_b2_mat3=sum(allele9q_b2_mat3);
count_allele10q_b2_mat3=sum(allele10q_b2_mat3);
count_allele11q_b2_mat3=sum(allele11q_b2_mat3);

freqallele1q_b2_mat3=sum(count_allele1q_b2_mat3)/(nobreeders2*2) ;
freqallele2q_b2_mat3=sum(count_allele2q_b2_mat3)/(nobreeders2*2) ;
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freqallele3q_b2_mat3=sum(count_allele3q_b2_mat3)/(nobreeders2*2) ;
freqallele4q_b2_mat3=sum(count_allele4q_b2_mat3)/(nobreeders2*2) ;
freqallele5q_b2_mat3=sum(count_allele5q_b2_mat3)/(nobreeders2*2) ;
freqallele6q_b2_mat3=sum(count_allele6q_b2_mat3)/(nobreeders2*2) ;
freqallele7q_b2_mat3=sum(count_allele7q_b2_mat3)/(nobreeders2*2) ;
freqallele8q_b2_mat3=sum(count_allele8q_b2_mat3)/(nobreeders2*2) ;
freqallele9q_b2_mat3=sum(count_allele9q_b2_mat3)/(nobreeders2*2) ;
freqallele10q_b2_mat3=sum(count_allele10q_b2_mat3)/(nobreeders2*2) ;
freqallele11q_b2_mat3=sum(count_allele11q_b2_mat3)/(nobreeders2*2) ;


dist3=rand(totalrowsb3,2) ;

allele1q_b2_mat4=(dist3>0) & (dist3<freqallele1_G1) ;
allele2q_b2_mat4=(dist3>=freqallele1_G1) &
(dist3<(freqallele1_G1+freqallele2_G1)) ;
allele3q_b2_mat4=(dist3>=(freqallele1_G1+freqallele2_G1)) &
(dist3<(freqallele1_G1+freqallele2_G1+freqallele3_G1)) ;
allele4q_b2_mat4=(dist3>=(freqallele1_G1+freqallele2_G1+freqallele3_G1)) &
(dist3<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1));
allele5q_b2_mat4=(dist3>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1)) &
(dist3<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1)) ;
allele6q_b2_mat4=(dist3>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1)) &
(dist3<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1)) ;
allele7q_b2_mat4=(dist3>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1+freqallele6_G1)) &
(dist3<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1));
allele8q_b2_mat4=(dist3>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1)) &
(dist3<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1)) ;
allele9q_b2_mat4=(dist3>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fre
qallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1))
&
(dist3<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+freqallele9_G1)) ;
allele10q_b2_mat4=(dist3>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fr
eqallele4_G1+freqallele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+f
reqallele9_G1)) &
(dist3<(freqallele1_G1+freqallele2_G1+freqallele3_G1+freqallele4_G1+freqal
lele5_G1+freqallele6_G1+freqallele7_G1+freqallele8_G1+freqallele9_G1+freqa
llele10_G1));
allele11q_b2_mat4=(dist3>=(freqallele1_G1+freqallele2_G1+freqallele3_G1+fr
eqallele4_G1+freqallele5_G1+freqallele6_G1++freqallele7_G1+freqallele8_G1+
freqallele9_G1+freqallele10_G1)) & (dist3<1) ;

allele1q_b2_mat4(all(allele1q_b2_mat4==1,2),:)=[];
allele2q_b2_mat4(all(allele2q_b2_mat4==1,2),:)=[];
allele3q_b2_mat4(all(allele3q_b2_mat4==1,2),:)=[];
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allele4q_b2_mat4(all(allele4q_b2_mat4==1,2),:)=[];
allele5q_b2_mat4(all(allele5q_b2_mat4==1,2),:)=[];
allele6q_b2_mat4(all(allele6q_b2_mat4==1,2),:)=[];
allele7q_b2_mat4(all(allele7q_b2_mat4==1,2),:)=[];
allele8q_b2_mat4(all(allele8q_b2_mat4==1,2),:)=[];
allele9q_b2_mat4(all(allele9q_b2_mat4==1,2),:)=[];
allele10q_b2_mat4(all(allele10q_b2_mat4==1,2),:)=[];
allele11q_b2_mat4(all(allele11q_b2_mat4==1,2),:)=[];

rows34=totalrowsb3-length(allele1q_b2_mat4(:,1)) ;
rows35=totalrowsb3-length(allele2q_b2_mat4(:,1)) ;
rows36=totalrowsb3-length(allele3q_b2_mat4(:,1)) ;
rows37=totalrowsb3-length(allele4q_b2_mat4(:,1)) ;
rows38=totalrowsb3-length(allele5q_b2_mat4(:,1)) ;
rows39=totalrowsb3-length(allele6q_b2_mat4(:,1)) ;
rows40=totalrowsb3-length(allele7q_b2_mat4(:,1)) ;
rows41=totalrowsb3-length(allele8q_b2_mat4(:,1));
rows42=totalrowsb3-length(allele9q_b2_mat4(:,1)) ;
rows43=totalrowsb3-length(allele10q_b2_mat4(:,1)) ;
rows44=totalrowsb3-length(allele11q_b2_mat4(:,1));

totalrowsb4=(rows34+rows35+rows36+rows37+rows38+rows39+rows40+rows41+rows4
2+rows43+rows44);

count_allele1q_b2_mat4=sum(allele1q_b2_mat4) ;
count_allele2q_b2_mat4=sum(allele2q_b2_mat4) ;
count_allele3q_b2_mat4=sum(allele3q_b2_mat4) ;
count_allele4q_b2_mat4=sum(allele4q_b2_mat4) ;
count_allele5q_b2_mat4=sum(allele5q_b2_mat4) ;
count_allele6q_b2_mat4=sum(allele6q_b2_mat4) ;
count_allele7q_b2_mat4=sum(allele7q_b2_mat4) ;
count_allele8q_b2_mat4=sum(allele8q_b2_mat4) ;
count_allele9q_b2_mat4=sum(allele9q_b2_mat4) ;
count_allele10q_b2_mat4=sum(allele10q_b2_mat4) ;
count_allele11q_b2_mat4=sum(allele11q_b2_mat4) ;

freqallele1q_b2_mat4=sum(count_allele1q_b2_mat4)/(nobreeders2*2);
freqallele2q_b2_mat4=sum(count_allele2q_b2_mat4)/(nobreeders2*2);
freqallele3q_b2_mat4=sum(count_allele3q_b2_mat4)/(nobreeders2*2) ;
freqallele4q_b2_mat4=sum(count_allele4q_b2_mat4)/(nobreeders2*2) ;
freqallele5q_b2_mat4=sum(count_allele5q_b2_mat4)/(nobreeders2*2) ;
freqallele6q_b2_mat4=sum(count_allele6q_b2_mat4)/(nobreeders2*2) ;
freqallele7q_b2_mat4=sum(count_allele7q_b2_mat4)/(nobreeders2*2) ;
freqallele8q_b2_mat4=sum(count_allele8q_b2_mat4)/(nobreeders2*2) ;
freqallele9q_b2_mat4=sum(count_allele9q_b2_mat4)/(nobreeders2*2) ;
freqallele10q_b2_mat4=sum(count_allele10q_b2_mat4)/(nobreeders2*2) ;
freqallele11q_b2_mat4=sum(count_allele11q_b2_mat4)/(nobreeders2*2) ;

freqallele1q_b2=freqallele1q_b2_mat1+freqallele1q_b2_mat2+freqallele1q_b2_
mat3+freqallele1q_b2_mat4 ;
freqallele2q_b2=freqallele2q_b2_mat1+freqallele2q_b2_mat2+freqallele2q_b2_
mat3+freqallele2q_b2_mat4 ;
freqallele3q_b2=freqallele3q_b2_mat1+freqallele3q_b2_mat2+freqallele3q_b2_
mat3+freqallele3q_b2_mat4 ;
```

```
freqallele4q_b2=freqallele4q_b2_mat1+freqallele4q_b2_mat2+freqallele4q_b2_
mat3+freqallele4q_b2_mat4 ;
freqallele5q_b2=freqallele5q_b2_mat1+freqallele5q_b2_mat2+freqallele5q_b2_
mat3+freqallele6q_b2_mat4 ;
freqallele6q_b2=freqallele6q_b2_mat1+freqallele6q_b2_mat2+freqallele6q_b2_
mat3+freqallele7q_b2_mat4 ;
freqallele7q_b2=freqallele7q_b2_mat1+freqallele7q_b2_mat2+freqallele7q_b2_
mat3+freqallele7q_b2_mat4 ;
freqallele8q_b2=freqallele8q_b2_mat1+freqallele8q_b2_mat2+freqallele8q_b2_
mat3+freqallele8q_b2_mat4 ;
freqallele9q_b2=freqallele9q_b2_mat1+freqallele9q_b2_mat2+freqallele9q_b2_
mat3+freqallele9q_b2_mat4 ;
freqallele10q_b2=freqallele10q_b2_mat1+freqallele10q_b2_mat2+freqallele10q
_b2_mat3+freqallele10q_b2_mat4 ;
freqallele11q_b2=freqallele11q_b2_mat1+freqallele11q_b2_mat2+freqallele11q
_b2_mat3+freqallele11q_b2_mat4 ;

%Frequency of breeder contributions to next generation includes the drones
%they mate with

%These (this) queen genotype(s) had mated with (n) drones in last
generation
%Hence breeder contribution is

Ballelefreq1_G2=(2*freqallele1q_b2+freqallele1d_G1)/3
Ballelefreq2_G2=(2*freqallele2q_b2+freqallele2d_G1)/3
Ballelefreq3_G2=(2*freqallele3q_b2+freqallele3d_G1)/3
Ballelefreq4_G2=(2*freqallele4q_b2+freqallele4d_G1)/3
Ballelefreq5_G2=(2*freqallele5q_b2+freqallele5d_G1)/3
Ballelefreq6_G2=(2*freqallele6q_b2+freqallele6d_G1)/3
Ballelefreq7_G2=(2*freqallele7q_b2+freqallele7d_G1)/3
Ballelefreq8_G2=(2*freqallele8q_b2+freqallele8d_G1)/3
Ballelefreq9_G2=(2*freqallele9q_b2+freqallele9d_G1)/3
Ballelefreq10_G2=(2*freqallele10q_b2+freqallele10d_G1)/3
Ballelefreq11_G2=(2*freqallele11q_b2+freqallele11d_G1)/3

%NOW USE ABOVE DISTRIBUTION TO GENERATE 100 QUEEN FREQUENY DIST. AND
%COMBINE WITH DRONE FREQUENCIES FROM BREEDER 1 TO CREATE G2 DIST

DistGen2Q=rand(nqueens,2);

allele1q_G2_mat1=(DistGen2Q>0) & (DistGen2Q<Ballelefreq1_G2);
allele2q_G2_mat1=(DistGen2Q>=Ballelefreq1_G2) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2));
allele3q_G2_mat1=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2));
allele4q_G2_mat1=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3
_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2));
allele5q_G2_mat1=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3
_G2+Ballelefreq4_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2));
```

```
allele6q_G2_mat1=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3
_G2+Ballelefreq4_G2+Ballelefreq5_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2));
allele7q_G2_mat1=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3
_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2));
allele8q_G2_mat1=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3
_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2));
allele9q_G2_mat1=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3
_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballel
efreq8_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballelef
req9_G2));
allele10q_G2_mat1=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq
3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Balle
lefreq8_G2+Ballelefreq9_G2)) &
(DistGen2Q<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G
2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballelef
req9_G2+Ballelefreq10_G2));
allele11q_G2_mat1=(DistGen2Q>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq
3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Balle
lefreq8_G2+Ballelefreq9_G2+Ballelefreq10_G2)) & (DistGen2Q<1);

allele1q_G2_mat1(all(allele1q_G2_mat1==1,2),:)=[] ;
allele2q_G2_mat1(all(allele2q_G2_mat1==1,2),:)=[] ;
allele3q_G2_mat1(all(allele3q_G2_mat1==1,2),:)=[] ;
allele4q_G2_mat1(all(allele4q_G2_mat1==1,2),:)=[] ;
allele5q_G2_mat1(all(allele5q_G2_mat1==1,2),:)=[] ;
allele6q_G2_mat1(all(allele6q_G2_mat1==1,2),:)=[] ;
allele7q_G2_mat1(all(allele7q_G2_mat1==1,2),:)=[] ;
allele8q_G2_mat1(all(allele8q_G2_mat1==1,2),:)=[] ;
allele9q_G2_mat1(all(allele9q_G2_mat1==1,2),:)=[] ;
allele10q_G2_mat1(all(allele10q_G2_mat1==1,2),:)=[] ;
allele11q_G2_mat1(all(allele11q_G2_mat1==1,2),:)=[] ;

rows1=nqueens-length(allele1q_G2_mat1(:,1)) ;
rows2=nqueens-length(allele2q_G2_mat1(:,1)) ;
rows3=nqueens-length(allele3q_G2_mat1(:,1)) ;
rows4=nqueens-length(allele4q_G2_mat1(:,1)) ;
rows5=nqueens-length(allele5q_G2_mat1(:,1)) ;
rows6=nqueens-length(allele6q_G2_mat1(:,1)) ;
rows7=nqueens-length(allele7q_G2_mat1(:,1)) ;
rows8=nqueens-length(allele8q_G2_mat1(:,1))  ;
rows9=nqueens-length(allele9q_G2_mat1(:,1)) ;
rows10=nqueens-length(allele10q_G2_mat1(:,1)) ;
rows11=nqueens-length(allele11q_G2_mat1(:,1))  ;

totalrows=(rows1+rows2+rows3+rows4+rows5+rows6+rows7+rows8+rows9+rows10+ro
ws11);
```

```
count_allele1q_G2_mat1=sum(allele1q_G2_mat1) ;
count_allele2q_G2_mat1=sum(allele2q_G2_mat1) ;
count_allele3q_G2_mat1=sum(allele3q_G2_mat1) ;
count_allele4q_G2_mat1=sum(allele4q_G2_mat1) ;
count_allele5q_G2_mat1=sum(allele5q_G2_mat1) ;
count_allele6q_G2_mat1=sum(allele6q_G2_mat1) ;
count_allele7q_G2_mat1=sum(allele7q_G2_mat1) ;
count_allele8q_G2_mat1=sum(allele8q_G2_mat1) ;
count_allele9q_G2_mat1=sum(allele9q_G2_mat1) ;
count_allele10q_G2_mat1=sum(allele10q_G2_mat1) ;
count_allele11q_G2_mat1=sum(allele11q_G2_mat1) ;

freqallele1q_G2_mat1=sum(count_allele1q_G2_mat1)/(nqueens*2) ;
freqallele2q_G2_mat1=sum(count_allele2q_G2_mat1)/(nqueens*2) ;
freqallele3q_G2_mat1=sum(count_allele3q_G2_mat1)/(nqueens*2) ;
freqallele4q_G2_mat1=sum(count_allele4q_G2_mat1)/(nqueens*2) ;
freqallele5q_G2_mat1=sum(count_allele5q_G2_mat1)/(nqueens*2) ;
freqallele6q_G2_mat1=sum(count_allele6q_G2_mat1)/(nqueens*2) ;
freqallele7q_G2_mat1=sum(count_allele7q_G2_mat1)/(nqueens*2) ;
freqallele8q_G2_mat1=sum(count_allele8q_G2_mat1)/(nqueens*2) ;
freqallele9q_G2_mat1=sum(count_allele9q_G2_mat1)/(nqueens*2) ;
freqallele10q_G2_mat1=sum(count_allele10q_G2_mat1)/(nqueens*2) ;
freqallele11q_G2_mat1=sum(count_allele11q_G2_mat1)/(nqueens*2) ;

DistGen2Q_2=rand(totalrows,2) ;

allele1q_G2_mat2=(DistGen2Q_2>0) & (DistGen2Q_2<Ballelefreq1_G2);
allele2q_G2_mat2=(DistGen2Q_2>=Ballelefreq1_G2) &
(DistGen2Q_2<(Ballelefreq1_G2+Ballelefreq2_G2));
allele3q_G2_mat2=(DistGen2Q_2>=(Ballelefreq1_G2+Ballelefreq2_G2)) &
(DistGen2Q_2<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2));
allele4q_G2_mat2=(DistGen2Q_2>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2)) &
(DistGen2Q_2<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2));
allele5q_G2_mat2=(DistGen2Q_2>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2)) &
(DistGen2Q_2<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2));
allele6q_G2_mat2=(DistGen2Q_2>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2)) &
(DistGen2Q_2<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2));
allele7q_G2_mat2=(DistGen2Q_2>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2)) &
(DistGen2Q_2<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2));
allele8q_G2_mat2=(DistGen2Q_2>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2)) &
(DistGen2Q_2<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2));
allele9q_G2_mat2=(DistGen2Q_2>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ball
```

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elefreq8_G2)) &
(DistGen2Q_2<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballel
efreq9_G2));
allele10q_G2_mat2=(DistGen2Q_2>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefr
eq3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Bal
lelefreq8_G2+Ballelefreq9_G2)) &
(DistGen2Q_2<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballel
efreq9_G2+Ballelefreq10_G2));
allele11q_G2_mat2=(DistGen2Q_2>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefr
eq3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Bal
lelefreq8_G2+Ballelefreq9_G2+Ballelefreq10_G2)) & (DistGen2Q_2<1);

allele1q_G2_mat2(all(allele1q_G2_mat2==1,2),:)=[] ;
allele2q_G2_mat2(all(allele2q_G2_mat2==1,2),:)=[] ;
allele3q_G2_mat2(all(allele3q_G2_mat2==1,2),:)=[] ;
allele4q_G2_mat2(all(allele4q_G2_mat2==1,2),:)=[] ;
allele5q_G2_mat2(all(allele5q_G2_mat2==1,2),:)=[] ;
allele6q_G2_mat2(all(allele6q_G2_mat2==1,2),:)=[] ;
allele7q_G2_mat2(all(allele7q_G2_mat2==1,2),:)=[] ;
allele8q_G2_mat2(all(allele8q_G2_mat2==1,2),:)=[] ;
allele9q_G2_mat2(all(allele9q_G2_mat2==1,2),:)=[] ;
allele10q_G2_mat2(all(allele10q_G2_mat2==1,2),:)=[] ;
allele11q_G2_mat2(all(allele11q_G2_mat2==1,2),:)=[] ;

rows12=totalrows-length(allele1q_G2_mat2(:,1)) ;
rows13=totalrows-length(allele2q_G2_mat2(:,1)) ;
rows14=totalrows-length(allele3q_G2_mat2(:,1)) ;
rows15=totalrows-length(allele4q_G2_mat2(:,1)) ;
rows16=totalrows-length(allele5q_G2_mat2(:,1)) ;
rows17=totalrows-length(allele6q_G2_mat2(:,1)) ;
rows18=totalrows-length(allele7q_G2_mat2(:,1)) ;
rows19=totalrows-length(allele8q_G2_mat2(:,1))  ;
rows20=totalrows-length(allele9q_G2_mat2(:,1)) ;
rows21=totalrows-length(allele10q_G2_mat2(:,1)) ;
rows22=totalrows-length(allele11q_G2_mat2(:,1))  ;

totalrows2=(rows12+rows13+rows14+rows15+rows16+rows17+rows18+rows19+rows20
+rows21+rows22);

count_allele1q_G2_mat2=sum(allele1q_G2_mat2) ;
count_allele2q_G2_mat2=sum(allele2q_G2_mat2) ;
count_allele3q_G2_mat2=sum(allele3q_G2_mat2) ;
count_allele4q_G2_mat2=sum(allele4q_G2_mat2) ;
count_allele5q_G2_mat2=sum(allele5q_G2_mat2) ;
count_allele6q_G2_mat2=sum(allele6q_G2_mat2) ;
count_allele7q_G2_mat2=sum(allele7q_G2_mat2) ;
count_allele8q_G2_mat2=sum(allele8q_G2_mat2) ;
count_allele9q_G2_mat2=sum(allele9q_G2_mat2) ;
count_allele10q_G2_mat2=sum(allele10q_G2_mat2) ;
count_allele11q_G2_mat2=sum(allele11q_G2_mat2) ;

freqallele1q_G2_mat2=sum(count_allele1q_G2_mat2)/(nqueens*2) ;
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```
freqallele2q_G2_mat2=sum(count_allele2q_G2_mat2)/(nqueens*2) ;
freqallele3q_G2_mat2=sum(count_allele3q_G2_mat2)/(nqueens*2) ;
freqallele4q_G2_mat2=sum(count_allele4q_G2_mat2)/(nqueens*2) ;
freqallele5q_G2_mat2=sum(count_allele5q_G2_mat2)/(nqueens*2) ;
freqallele6q_G2_mat2=sum(count_allele6q_G2_mat2)/(nqueens*2) ;
freqallele7q_G2_mat2=sum(count_allele7q_G2_mat2)/(nqueens*2) ;
freqallele8q_G2_mat2=sum(count_allele8q_G2_mat2)/(nqueens*2) ;
freqallele9q_G2_mat2=sum(count_allele9q_G2_mat2)/(nqueens*2) ;
freqallele10q_G2_mat2=sum(count_allele10q_G2_mat2)/(nqueens*2) ;
freqallele11q_G2_mat2=sum(count_allele11q_G2_mat2)/(nqueens*2) ;

DistGen2Q_3=rand(totalrows2,2);

allele1q_G2_mat3=(DistGen2Q_3>0) & (DistGen2Q_3<Ballelefreq1_G2);
allele2q_G2_mat3=(DistGen2Q_3>=Ballelefreq1_G2) &
(DistGen2Q_3<(Ballelefreq1_G2+Ballelefreq2_G2));
allele3q_G2_mat3=(DistGen2Q_3>=(Ballelefreq1_G2+Ballelefreq2_G2)) &
(DistGen2Q_3<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2));
allele4q_G2_mat3=(DistGen2Q_3>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2)) &
(DistGen2Q_3<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2));
allele5q_G2_mat3=(DistGen2Q_3>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2)) &
(DistGen2Q_3<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2));
allele6q_G2_mat3=(DistGen2Q_3>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2)) &
(DistGen2Q_3<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2));
allele7q_G2_mat3=(DistGen2Q_3>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2)) &
(DistGen2Q_3<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2));
allele8q_G2_mat3=(DistGen2Q_3>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2)) &
(DistGen2Q_3<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2));
allele9q_G2_mat3=(DistGen2Q_3>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ball
elefreq8_G2)) &
(DistGen2Q_3<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballel
efreq9_G2));
allele10q_G2_mat3=(DistGen2Q_3>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefr
eq3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Bal
lelefreq8_G2+Ballelefreq9_G2)) &
(DistGen2Q_3<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballel
efreq9_G2+Ballelefreq10_G2));
allele11q_G2_mat3=(DistGen2Q_3>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefr
eq3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Bal
lelefreq8_G2+Ballelefreq9_G2+Ballelefreq10_G2)) & (DistGen2Q_3<1);

allele1q_G2_mat3(all(allele1q_G2_mat3==1,2),:)=[] ;
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```
allele2q_G2_mat3(all(allele2q_G2_mat3==1,2),:)=[] ;
allele3q_G2_mat3(all(allele3q_G2_mat3==1,2),:)=[] ;
allele4q_G2_mat3(all(allele4q_G2_mat3==1,2),:)=[] ;
allele5q_G2_mat3(all(allele5q_G2_mat3==1,2),:)=[] ;
allele6q_G2_mat3(all(allele6q_G2_mat3==1,2),:)=[] ;
allele7q_G2_mat3(all(allele7q_G2_mat3==1,2),:)=[] ;
allele8q_G2_mat3(all(allele8q_G2_mat3==1,2),:)=[] ;
allele9q_G2_mat3(all(allele9q_G2_mat3==1,2),:)=[] ;
allele10q_G2_mat3(all(allele10q_G2_mat3==1,2),:)=[] ;
allele11q_G2_mat3(all(allele11q_G2_mat3==1,2),:)=[] ;

rows23=totalrows2-length(allele1q_G2_mat3(:,1)) ;
rows24=totalrows2-length(allele2q_G2_mat3(:,1)) ;
rows25=totalrows2-length(allele3q_G2_mat3(:,1)) ;
rows26=totalrows2-length(allele4q_G2_mat3(:,1)) ;
rows27=totalrows2-length(allele5q_G2_mat3(:,1)) ;
rows28=totalrows2-length(allele6q_G2_mat3(:,1)) ;
rows29=totalrows2-length(allele7q_G2_mat3(:,1)) ;
rows30=totalrows2-length(allele8q_G2_mat3(:,1))  ;
rows31=totalrows2-length(allele9q_G2_mat3(:,1)) ;
rows32=totalrows2-length(allele10q_G2_mat3(:,1)) ;
rows33=totalrows2-length(allele11q_G2_mat3(:,1))  ;

totalrows3=(rows23+rows24+rows25+rows26+rows27+rows28+rows29+rows30+rows31
+rows32+rows33);

count_allele1q_G2_mat3=sum(allele1q_G2_mat3) ;
count_allele2q_G2_mat3=sum(allele2q_G2_mat3) ;
count_allele3q_G2_mat3=sum(allele3q_G2_mat3) ;
count_allele4q_G2_mat3=sum(allele4q_G2_mat3) ;
count_allele5q_G2_mat3=sum(allele5q_G2_mat3) ;
count_allele6q_G2_mat3=sum(allele6q_G2_mat3) ;
count_allele7q_G2_mat3=sum(allele7q_G2_mat3) ;
count_allele8q_G2_mat3=sum(allele8q_G2_mat3) ;
count_allele9q_G2_mat3=sum(allele9q_G2_mat3) ;
count_allele10q_G2_mat3=sum(allele10q_G2_mat3) ;
count_allele11q_G2_mat3=sum(allele11q_G2_mat3) ;

freqallele1q_G2_mat3=sum(count_allele1q_G2_mat3)/(nqueens*2) ;
freqallele2q_G2_mat3=sum(count_allele2q_G2_mat3)/(nqueens*2) ;
freqallele3q_G2_mat3=sum(count_allele3q_G2_mat3)/(nqueens*2) ;
freqallele4q_G2_mat3=sum(count_allele4q_G2_mat3)/(nqueens*2) ;
freqallele5q_G2_mat3=sum(count_allele5q_G2_mat3)/(nqueens*2) ;
freqallele6q_G2_mat3=sum(count_allele6q_G2_mat3)/(nqueens*2) ;
freqallele7q_G2_mat3=sum(count_allele7q_G2_mat3)/(nqueens*2) ;
freqallele8q_G2_mat3=sum(count_allele8q_G2_mat3)/(nqueens*2) ;
freqallele9q_G2_mat3=sum(count_allele9q_G2_mat3)/(nqueens*2) ;
freqallele10q_G2_mat3=sum(count_allele10q_G2_mat3)/(nqueens*2) ;
freqallele11q_G2_mat3=sum(count_allele11q_G2_mat3)/(nqueens*2) ;

DistGen2Q_4=rand(totalrows3,2) ;

allele1q_G2_mat4=(DistGen2Q_4>0) & (DistGen2Q_4<Ballelefreq1_G2);
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allele2q_G2_mat4=(DistGen2Q_4>=Ballelefreq1_G2) &
(DistGen2Q_4<(Ballelefreq1_G2+Ballelefreq2_G2));
allele3q_G2_mat4=(DistGen2Q_4>=(Ballelefreq1_G2+Ballelefreq2_G2)) &
(DistGen2Q_4<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2));
allele4q_G2_mat4=(DistGen2Q_4>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2)) &
(DistGen2Q_4<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2));
allele5q_G2_mat4=(DistGen2Q_4>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2)) &
(DistGen2Q_4<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2));
allele6q_G2_mat4=(DistGen2Q_4>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2)) &
(DistGen2Q_4<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2));
allele7q_G2_mat4=(DistGen2Q_4>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2)) &
(DistGen2Q_4<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2));
allele8q_G2_mat4=(DistGen2Q_4>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2)) &
(DistGen2Q_4<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2));
allele9q_G2_mat4=(DistGen2Q_4>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ball
elefreq8_G2)) &
(DistGen2Q_4<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballel
efreq9_G2));
allele10q_G2_mat4=(DistGen2Q_4>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefr
eq3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Bal
lelefreq8_G2+Ballelefreq9_G2)) &
(DistGen2Q_4<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballel
efreq9_G2+Ballelefreq10_G2));
allele11q_G2_mat4=(DistGen2Q_4>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefr
eq3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Bal
lelefreq8_G2+Ballelefreq9_G2+Ballelefreq10_G2)) & (DistGen2Q_4<1);

allele1q_G2_mat4(all(allele1q_G2_mat4==1,2),:)=[] ;
allele2q_G2_mat4(all(allele2q_G2_mat4==1,2),:)=[] ;
allele3q_G2_mat4(all(allele3q_G2_mat4==1,2),:)=[] ;
allele4q_G2_mat4(all(allele4q_G2_mat4==1,2),:)=[] ;
allele5q_G2_mat4(all(allele5q_G2_mat4==1,2),:)=[] ;
allele6q_G2_mat4(all(allele6q_G2_mat4==1,2),:)=[] ;
allele7q_G2_mat4(all(allele7q_G2_mat4==1,2),:)=[] ;
allele8q_G2_mat4(all(allele8q_G2_mat4==1,2),:)=[] ;
allele9q_G2_mat4(all(allele9q_G2_mat4==1,2),:)=[] ;
allele10q_G2_mat4(all(allele10q_G2_mat4==1,2),:)=[] ;
allele11q_G2_mat4(all(allele11q_G2_mat4==1,2),:)=[] ;

rows34=totalrows3-length(allele1q_G2_mat4(:,1)) ;
rows35=totalrows3-length(allele2q_G2_mat4(:,1)) ;
rows36=totalrows3-length(allele3q_G2_mat4(:,1)) ;
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rows37=totalrows3-length(allele4q_G2_mat4(:,1)) ;
rows38=totalrows3-length(allele5q_G2_mat4(:,1)) ;
rows39=totalrows3-length(allele6q_G2_mat4(:,1)) ;
rows40=totalrows3-length(allele7q_G2_mat4(:,1)) ;
rows41=totalrows3-length(allele8q_G2_mat4(:,1)) ;
rows42=totalrows3-length(allele9q_G2_mat4(:,1)) ;
rows43=totalrows3-length(allele10q_G2_mat4(:,1)) ;
rows44=totalrows3-length(allele11q_G2_mat4(:,1)) ;

totalrows4=(rows34+rows35+rows36+rows37+rows38+rows39+rows40+rows41+rows42
+rows43+rows44);


count_allele1q_G2_mat4=sum(allele1q_G2_mat4) ;
count_allele2q_G2_mat4=sum(allele2q_G2_mat4) ;
count_allele3q_G2_mat4=sum(allele3q_G2_mat4) ;
count_allele4q_G2_mat4=sum(allele4q_G2_mat4) ;
count_allele5q_G2_mat4=sum(allele5q_G2_mat4) ;
count_allele6q_G2_mat4=sum(allele6q_G2_mat4) ;
count_allele7q_G2_mat4=sum(allele7q_G2_mat4) ;
count_allele8q_G2_mat4=sum(allele8q_G2_mat4) ;
count_allele9q_G2_mat4=sum(allele9q_G2_mat4) ;
count_allele10q_G2_mat4=sum(allele10q_G2_mat4) ;
count_allele11q_G2_mat4=sum(allele11q_G2_mat4) ;

freqallele1q_G2_mat4=sum(count_allele1q_G2_mat4)/(nqueens*2) ;
freqallele2q_G2_mat4=sum(count_allele2q_G2_mat4)/(nqueens*2) ;
freqallele3q_G2_mat4=sum(count_allele3q_G2_mat4)/(nqueens*2) ;
freqallele4q_G2_mat4=sum(count_allele4q_G2_mat4)/(nqueens*2) ;
freqallele5q_G2_mat4=sum(count_allele5q_G2_mat4)/(nqueens*2) ;
freqallele6q_G2_mat4=sum(count_allele6q_G2_mat4)/(nqueens*2) ;
freqallele7q_G2_mat4=sum(count_allele7q_G2_mat4)/(nqueens*2) ;
freqallele8q_G2_mat4=sum(count_allele8q_G2_mat4)/(nqueens*2) ;
freqallele9q_G2_mat4=sum(count_allele9q_G2_mat4)/(nqueens*2) ;
freqallele10q_G2_mat4=sum(count_allele10q_G2_mat4)/(nqueens*2) ;
freqallele11q_G2_mat4=sum(count_allele11q_G2_mat4)/(nqueens*2) ;

DistGen2Q_5=rand(totalrows4,2) ;

allele1q_G2_mat5=(DistGen2Q_5>0) & (DistGen2Q_5<Ballelefreq1_G2);
allele2q_G2_mat5=(DistGen2Q_5>=Ballelefreq1_G2) &
(DistGen2Q_5<(Ballelefreq1_G2+Ballelefreq2_G2));
allele3q_G2_mat5=(DistGen2Q_5>=(Ballelefreq1_G2+Ballelefreq2_G2)) &
(DistGen2Q_5<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2));
allele4q_G2_mat5=(DistGen2Q_5>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2)) &
(DistGen2Q_5<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2));
allele5q_G2_mat5=(DistGen2Q_5>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2)) &
(DistGen2Q_5<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2));
allele6q_G2_mat5=(DistGen2Q_5>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2)) &
```

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(DistGen2Q_5<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2));
allele7q_G2_mat5=(DistGen2Q_5>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2)) &
(DistGen2Q_5<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2));
allele8q_G2_mat5=(DistGen2Q_5>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2)) &
(DistGen2Q_5<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2));
allele9q_G2_mat5=(DistGen2Q_5>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefre
q3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ball
elefreq8_G2)) &
(DistGen2Q_5<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballel
efreq9_G2));
allele10q_G2_mat5=(DistGen2Q_5>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefr
eq3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Bal
lelefreq8_G2+Ballelefreq9_G2)) &
(DistGen2Q_5<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4
_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballel
efreq9_G2+Ballelefreq10_G2));
allele11q_G2_mat5=(DistGen2Q_5>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefr
eq3_G2+Ballelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Bal
lelefreq8_G2+Ballelefreq9_G2+Ballelefreq10_G2)) & (DistGen2Q_5<1);

allele1q_G2_mat5(all(allele1q_G2_mat5==1,2),:)=[] ;
allele2q_G2_mat5(all(allele2q_G2_mat5==1,2),:)=[] ;
allele3q_G2_mat5(all(allele3q_G2_mat5==1,2),:)=[] ;
allele4q_G2_mat5(all(allele4q_G2_mat5==1,2),:)=[] ;
allele5q_G2_mat5(all(allele5q_G2_mat5==1,2),:)=[] ;
allele6q_G2_mat5(all(allele6q_G2_mat5==1,2),:)=[] ;
allele7q_G2_mat5(all(allele7q_G2_mat5==1,2),:)=[] ;
allele8q_G2_mat5(all(allele8q_G2_mat5==1,2),:)=[] ;
allele9q_G2_mat5(all(allele9q_G2_mat5==1,2),:)=[] ;
allele10q_G2_mat5(all(allele10q_G2_mat5==1,2),:)=[] ;
allele11q_G2_mat5(all(allele11q_G2_mat5==1,2),:)=[] ;

rows45=totalrows4-length(allele1q_G2_mat5(:,1)) ;
rows46=totalrows4-length(allele2q_G2_mat5(:,1)) ;
rows47=totalrows4-length(allele3q_G2_mat5(:,1)) ;
rows48=totalrows4-length(allele4q_G2_mat5(:,1)) ;
rows49=totalrows4-length(allele5q_G2_mat5(:,1)) ;
rows50=totalrows4-length(allele6q_G2_mat5(:,1)) ;
rows51=totalrows4-length(allele7q_G2_mat5(:,1)) ;
rows52=totalrows4-length(allele8q_G2_mat5(:,1)) ;
rows53=totalrows4-length(allele9q_G2_mat5(:,1)) ;
rows54=totalrows4-length(allele10q_G2_mat5(:,1)) ;
rows55=totalrows4-length(allele11q_G2_mat5(:,1)) ;

totalrows5=(rows45+rows46+rows47+rows48+rows49+rows50+rows51+rows52+rows53
+rows54+rows55);

count_allele1q_G2_mat5=sum(allele1q_G2_mat5) ;
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count_allele2q_G2_mat5=sum(allele2q_G2_mat5) ;
count_allele3q_G2_mat5=sum(allele3q_G2_mat5) ;
count_allele4q_G2_mat5=sum(allele4q_G2_mat5) ;
count_allele5q_G2_mat5=sum(allele5q_G2_mat5) ;
count_allele6q_G2_mat5=sum(allele6q_G2_mat5) ;
count_allele7q_G2_mat5=sum(allele7q_G2_mat5) ;
count_allele8q_G2_mat5=sum(allele8q_G2_mat5) ;
count_allele9q_G2_mat5=sum(allele9q_G2_mat5) ;
count_allele10q_G2_mat5=sum(allele10q_G2_mat5) ;
count_allele11q_G2_mat5=sum(allele11q_G2_mat5) ;

freqallele1q_G2_mat5=sum(count_allele1q_G2_mat5)/(nqueens*2) ;
freqallele2q_G2_mat5=sum(count_allele2q_G2_mat5)/(nqueens*2) ;
freqallele3q_G2_mat5=sum(count_allele3q_G2_mat5)/(nqueens*2) ;
freqallele4q_G2_mat5=sum(count_allele4q_G2_mat5)/(nqueens*2) ;
freqallele5q_G2_mat5=sum(count_allele5q_G2_mat5)/(nqueens*2) ;
freqallele6q_G2_mat5=sum(count_allele6q_G2_mat5)/(nqueens*2) ;
freqallele7q_G2_mat5=sum(count_allele7q_G2_mat5)/(nqueens*2) ;
freqallele8q_G2_mat5=sum(count_allele8q_G2_mat5)/(nqueens*2) ;
freqallele9q_G2_mat5=sum(count_allele9q_G2_mat5)/(nqueens*2) ;
freqallele10q_G2_mat5=sum(count_allele10q_G2_mat5)/(nqueens*2) ;
freqallele11q_G2_mat5=sum(count_allele11q_G2_mat5)/(nqueens*2) ;

freqallele1q_G2=freqallele1q_G2_mat1+freqallele1q_G2_mat2+freqallele1q_G2_
mat3+freqallele1q_G2_mat4+freqallele1q_G2_mat5;
freqallele2q_G2=freqallele2q_G2_mat1+freqallele2q_G2_mat2+freqallele2q_G2_
mat3+freqallele2q_G2_mat4+freqallele2q_G2_mat5;
freqallele3q_G2=freqallele3q_G2_mat1+freqallele3q_G2_mat2+freqallele3q_G2_
mat3+freqallele3q_G2_mat4+freqallele3q_G2_mat5;
freqallele4q_G2=freqallele4q_G2_mat1+freqallele4q_G2_mat2+freqallele4q_G2_
mat3+freqallele4q_G2_mat4+freqallele4q_G2_mat5;
freqallele5q_G2=freqallele5q_G2_mat1+freqallele5q_G2_mat2+freqallele5q_G2_
mat3+freqallele5q_G2_mat4+freqallele5q_G2_mat5;
freqallele6q_G2=freqallele6q_G2_mat1+freqallele6q_G2_mat2+freqallele6q_G2_
mat3+freqallele6q_G2_mat4+freqallele6q_G2_mat5;
freqallele7q_G2=freqallele7q_G2_mat1+freqallele7q_G2_mat2+freqallele7q_G2_
mat3+freqallele7q_G2_mat4+freqallele7q_G2_mat5;
freqallele8q_G2=freqallele8q_G2_mat1+freqallele8q_G2_mat2+freqallele8q_G2_
mat3+freqallele8q_G2_mat4+freqallele8q_G2_mat5;
freqallele9q_G2=freqallele9q_G2_mat1+freqallele9q_G2_mat2+freqallele9q_G2_
mat3+freqallele9q_G2_mat4+freqallele9q_G2_mat5;
freqallele10q_G2=freqallele10q_G2_mat1+freqallele10q_G2_mat2+freqallele10q
_G2_mat3+freqallele10q_G2_mat4+freqallele10q_G2_mat5;
freqallele11q_G2=freqallele11q_G2_mat1+freqallele11q_G2_mat2+freqallele11q
_G2_mat3+freqallele11q_G2_mat4+freqallele11q_G2_mat5;


freqallele1_G2=(2*freqallele1q_G2+freqallele1d_G1)/3;
freqallele2_G2=(2*freqallele2q_G2+freqallele2d_G1)/3;
freqallele3_G2=(2*freqallele3q_G2+freqallele3d_G1)/3;
freqallele4_G2=(2*freqallele4q_G2+freqallele4d_G1)/3;
freqallele5_G2=(2*freqallele5q_G2+freqallele5d_G1)/3;
freqallele6_G2=(2*freqallele6q_G2+freqallele6d_G1)/3;
freqallele7_G2=(2*freqallele7q_G2+freqallele7d_G1)/3;
```

```
freqallele8_G2=(2*freqallele8q_G2+freqallele8d_G1)/3;
freqallele9_G2=(2*freqallele9q_G2+freqallele9d_G1)/3;
freqallele10_G2=(2*freqallele10q_G2+freqallele10d_G1)/3;
freqallele11_G2=(2*freqallele11q_G2+freqallele11d_G1)/3;

mat1_G2(freqallele1)=freqallele1_G2
mat2_G2(freqallele1)=freqallele2_G2
mat3_G2(freqallele1)=freqallele3_G2
mat4_G2(freqallele1)=freqallele4_G2
mat5_G2(freqallele1)=freqallele5_G2
mat6_G2(freqallele1)=freqallele6_G2
mat7_G2(freqallele1)=freqallele7_G2
mat8_G2(freqallele1)=freqallele8_G2
mat9_G2(freqallele1)=freqallele9_G2
mat10_G2(freqallele1)=freqallele10_G2
mat11_G2(freqallele1)=freqallele11_G2









%THIRD GENERATION SIMULTION
%ALLELE FREQUENCIES CARRIED BY DRONES CONTRIBUTING TO NEXT GENERATION
COMES
%FROM QUEENS SELECTED AS BREEDERS LAST YEAR ie Ballelefreqx_G2


G2drones=rand(nqueens,nodrones) ;


allele1d_2=(G2drones>0) & (G2drones<Ballelefreq1_G2);
allele2d_2=(G2drones>=Ballelefreq1_G2) &
(G2drones<(Ballelefreq1_G2+Ballelefreq2_G2));
allele3d_2=(G2drones>=(Ballelefreq1_G2+Ballelefreq2_G2)) &
(G2drones<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2));
allele4d_2=(G2drones>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2)) &
(G2drones<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G2
));
allele5d_2=(G2drones>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Bal
lelefreq4_G2)) &
(G2drones<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G2
+Ballelefreq5_G2));
allele6d_2=(G2drones>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Bal
lelefreq4_G2+Ballelefreq5_G2)) &
(G2drones<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G2
+Ballelefreq5_G2+Ballelefreq6_G2));
allele7d_2=(G2drones>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Bal
lelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2)) &
```

```
(G2drones<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G2
+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2));
allele8d_2=(G2drones>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Bal
lelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2)) &
(G2drones<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G2
+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2));
allele9d_2=(G2drones>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Bal
lelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_
G2)) &
(G2drones<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G2
+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballelefr
eq9_G2));
allele10d_2=(G2drones>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ba
llelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8
_G2+Ballelefreq9_G2)) &
(G2drones<(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ballelefreq4_G2
+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8_G2+Ballelefr
eq9_G2+Ballelefreq10_G2));
allele11d_2=(G2drones>=(Ballelefreq1_G2+Ballelefreq2_G2+Ballelefreq3_G2+Ba
llelefreq4_G2+Ballelefreq5_G2+Ballelefreq6_G2+Ballelefreq7_G2+Ballelefreq8
_G2+Ballelefreq9_G2+Ballelefreq10_G2)) & (G2drones<1);

count_allele1d_2=sum(allele1d_2) ;
count_allele2d_2=sum(allele2d_2) ;
count_allele3d_2=sum(allele3d_2) ;
count_allele4d_2=sum(allele4d_2) ;
count_allele5d_2=sum(allele5d_2) ;
count_allele6d_2=sum(allele6d_2) ;
count_allele7d_2=sum(allele7d_2) ;
count_allele8d_2=sum(allele8d_2) ;
count_allele9d_2=sum(allele9d_2) ;
count_allele10d_2=sum(allele10d_2) ;
count_allele11d_2=sum(allele11d_2) ;

freqallele1d_G2=sum(count_allele1d_2)/(nqueens*nodrones);
freqallele2d_G2=sum(count_allele2d_2)/(nqueens*nodrones);
freqallele3d_G2=sum(count_allele3d_2)/(nqueens*nodrones);
freqallele4d_G2=sum(count_allele4d_2)/(nqueens*nodrones);
freqallele5d_G2=sum(count_allele5d_2)/(nqueens*nodrones);
freqallele6d_G2=sum(count_allele6d_2)/(nqueens*nodrones);
freqallele7d_G2=sum(count_allele7d_2)/(nqueens*nodrones);
freqallele8d_G2=sum(count_allele8d_2)/(nqueens*nodrones);
freqallele9d_G2=sum(count_allele9d_2)/(nqueens*nodrones);
freqallele10d_G2=sum(count_allele10d_2)/(nqueens*nodrones);
freqallele11d_G2=sum(count_allele11d_2)/(nqueens*nodrones);




%SELECT BREEDERS FROM THE G2 DISTRIBUTION
DistG2=rand(nqueens,2) ;
Breeders3=datasample(DistG2,nobreeders3) ;

allele1q_b3_mat1=(Breeders3>0) & (Breeders3<freqallele1_G2) ;
```

```
allele2q_b3_mat1=(Breeders3>=freqallele1_G2) &
(Breeders3<(freqallele1_G2+freqallele2_G2)) ;
allele3q_b3_mat1=(Breeders3>=(freqallele1_G2+freqallele2_G2)) &
(Breeders3<(freqallele1_G2+freqallele2_G2+freqallele3_G2)) ;
allele4q_b3_mat1=(Breeders3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2
)) &
(Breeders3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2))
;
allele5q_b3_mat1=(Breeders3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2
+freqallele4_G2)) &
(Breeders3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+fr
eqallele5_G2)) ;
allele6q_b3_mat1=(Breeders3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2
+freqallele4_G2+freqallele5_G2)) &
(Breeders3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+fr
eqallele5_G2+freqallele6_G2)) ;
allele7q_b3_mat1=(Breeders3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2
+freqallele4_G2+freqallele5_G2+freqallele6_G2)) &
(Breeders3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+fr
eqallele5_G2+freqallele6_G2+freqallele7_G2)) ;
allele8q_b3_mat1=(Breeders3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2
+freqallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2)) &
(Breeders3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+fr
eqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2)) ;
allele9q_b3_mat1=(Breeders3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2
+freqallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G
2)) &
(Breeders3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+fr
eqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+freqallele9_G2))
;
allele10q_b3_mat1=(Breeders3>=(freqallele1_G2+freqallele2_G2+freqallele3_G
2+freqallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_
G2+freqallele9_G2)) &
(Breeders3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+fr
eqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+freqallele9_G2+f
reqallele10_G2)) ;
allele11q_b3_mat1=(Breeders3>=(freqallele1_G2+freqallele2_G2+freqallele3_G
2+freqallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_
G2+freqallele9_G2+freqallele10_G2)) & (Breeders3<1) ;

allele1q_b3_mat1(all(allele1q_b3_mat1==1,2),:)=[];
allele2q_b3_mat1(all(allele2q_b3_mat1==1,2),:)=[];
allele3q_b3_mat1(all(allele3q_b3_mat1==1,2),:)=[];
allele4q_b3_mat1(all(allele4q_b3_mat1==1,2),:)=[];
allele5q_b3_mat1(all(allele5q_b3_mat1==1,2),:)=[];
allele6q_b3_mat1(all(allele6q_b3_mat1==1,2),:)=[];
allele7q_b3_mat1(all(allele7q_b3_mat1==1,2),:)=[];
allele8q_b3_mat1(all(allele8q_b2_mat1==1,2),:)=[];
allele9q_b3_mat1(all(allele9q_b3_mat1==1,2),:)=[];
allele10q_b3_mat1(all(allele10q_b3_mat1==1,2),:)=[];
allele11q_b3_mat1(all(allele11q_b2_mat1==1,2),:)=[];

rows1=nobreeders3-length(allele1q_b3_mat1(:,1));
rows2=nobreeders3-length(allele2q_b3_mat1(:,1));
rows3=nobreeders3-length(allele3q_b3_mat1(:,1));
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rows4=nobreeders3-length(allele4q_b3_mat1(:,1));
rows5=nobreeders3-length(allele5q_b3_mat1(:,1));
rows6=nobreeders3-length(allele6q_b3_mat1(:,1));
rows7=nobreeders3-length(allele7q_b3_mat1(:,1));
rows8=nobreeders3-length(allele8q_b3_mat1(:,1));
rows9=nobreeders3-length(allele6q_b3_mat1(:,1));
rows10=nobreeders3-length(allele7q_b3_mat1(:,1));
rows11=nobreeders3-length(allele8q_b3_mat1(:,1));
totalrowsb1=(rows1+rows2+rows3+rows4+rows5+rows6+rows7+rows8+rows9+rows10+
rows11);

count_allele1q_b3_mat1=sum(allele1q_b3_mat1);
count_allele2q_b3_mat1=sum(allele2q_b3_mat1);
count_allele3q_b3_mat1=sum(allele3q_b3_mat1);
count_allele4q_b3_mat1=sum(allele4q_b3_mat1);
count_allele5q_b3_mat1=sum(allele5q_b3_mat1);
count_allele6q_b3_mat1=sum(allele6q_b3_mat1);
count_allele7q_b3_mat1=sum(allele7q_b3_mat1);
count_allele8q_b3_mat1=sum(allele8q_b3_mat1);
count_allele9q_b3_mat1=sum(allele9q_b3_mat1);
count_allele10q_b3_mat1=sum(allele10q_b3_mat1);
count_allele11q_b3_mat1=sum(allele11q_b3_mat1);

freqallele1q_b3_mat1=sum(count_allele1q_b3_mat1)/(nobreeders3*2);
freqallele2q_b3_mat1=sum(count_allele2q_b3_mat1)/(nobreeders3*2);
freqallele3q_b3_mat1=sum(count_allele3q_b3_mat1)/(nobreeders3*2);
freqallele4q_b3_mat1=sum(count_allele4q_b3_mat1)/(nobreeders3*2);
freqallele5q_b3_mat1=sum(count_allele5q_b3_mat1)/(nobreeders3*2);
freqallele6q_b3_mat1=sum(count_allele6q_b3_mat1)/(nobreeders3*2);
freqallele7q_b3_mat1=sum(count_allele7q_b3_mat1)/(nobreeders3*2);
freqallele8q_b3_mat1=sum(count_allele8q_b3_mat1)/(nobreeders3*2);
freqallele9q_b3_mat1=sum(count_allele9q_b3_mat1)/(nobreeders3*2);
freqallele10q_b3_mat1=sum(count_allele10q_b3_mat1)/(nobreeders3*2);
freqallele11q_b3_mat1=sum(count_allele11q_b3_mat1)/(nobreeders3*2);

dist1=rand(totalrowsb1,2) ;

allele1q_b3_mat2=(dist1>0) & (dist1<freqallele1_G2) ;
allele2q_b3_mat2=(dist1>=freqallele1_G2) &
(dist1<(freqallele1_G2+freqallele2_G2)) ;
allele3q_b3_mat2=(dist1>=(freqallele1_G2+freqallele2_G2)) &
(dist1<(freqallele1_G2+freqallele2_G2+freqallele3_G2)) ;
allele4q_b3_mat2=(dist1>=(freqallele1_G2+freqallele2_G2+freqallele3_G2)) &
(dist1<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2)) ;
allele5q_b3_mat2=(dist1>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2)) &
(dist1<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2)) ;
allele6q_b3_mat2=(dist1>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2)) &
(dist1<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2)) ;
allele7q_b3_mat2=(dist1>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2+freqallele6_G2)) &
```

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(dist1<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2)) ;
allele8q_b3_mat2=(dist1>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2)) &
(dist1<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2)) ;
allele9q_b3_mat2=(dist1>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2))
&
(dist1<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+freqallele9_G2)) ;
allele10q_b3_mat2=(dist1>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fr
eqallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+f
reqallele9_G2)) &
(dist1<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+freqallele9_G2+freqa
llele10_G2)) ;
allele11q_b3_mat2=(dist1>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fr
eqallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+f
reqallele9_G2+freqallele10_G2)) & (dist1<1) ;

allele1q_b3_mat2(all(allele1q_b3_mat2==1,2),:)=[] ;
allele2q_b3_mat2(all(allele2q_b3_mat2==1,2),:)=[] ;
allele3q_b3_mat2(all(allele3q_b3_mat2==1,2),:)=[] ;
allele4q_b3_mat2(all(allele4q_b3_mat2==1,2),:)=[] ;
allele5q_b3_mat2(all(allele5q_b3_mat2==1,2),:)=[] ;
allele6q_b3_mat2(all(allele6q_b3_mat2==1,2),:)=[] ;
allele7q_b3_mat2(all(allele7q_b3_mat2==1,2),:)=[] ;
allele8q_b3_mat2(all(allele8q_b3_mat2==1,2),:)=[] ;
allele9q_b3_mat2(all(allele8q_b3_mat2==1,2),:)=[] ;
allele10q_b3_mat2(all(allele10q_b3_mat2==1,2),:)=[] ;
allele11q_b3_mat2(all(allele11q_b3_mat2==1,2),:)=[] ;

rows12=totalrowsb1-length(allele1q_b3_mat2(:,1)) ;
rows13=totalrowsb1-length(allele2q_b3_mat2(:,1)) ;
rows14=totalrowsb1-length(allele3q_b3_mat2(:,1));
rows15=totalrowsb1-length(allele4q_b3_mat2(:,1));
rows16=totalrowsb1-length(allele5q_b3_mat2(:,1)) ;
rows17=totalrowsb1-length(allele6q_b3_mat2(:,1)) ;
rows18=totalrowsb1-length(allele7q_b3_mat2(:,1)) ;
rows19=totalrowsb1-length(allele8q_b3_mat2(:,1)) ;
rows20=totalrowsb1-length(allele9q_b3_mat2(:,1)) ;
rows21=totalrowsb1-length(allele10q_b3_mat2(:,1)) ;
rows22=totalrowsb1-length(allele11q_b3_mat2(:,1)) ;

totalrowsb2=(rows12+rows13+rows14+rows15+rows16+rows17+rows18+rows19+rows2
0+rows21+rows22);

count_allele1q_b3_mat2=sum(allele1q_b3_mat2) ;
count_allele2q_b3_mat2=sum(allele2q_b3_mat2) ;
count_allele3q_b3_mat2=sum(allele3q_b3_mat2) ;
count_allele4q_b3_mat2=sum(allele4q_b3_mat2) ;
count_allele5q_b3_mat2=sum(allele5q_b3_mat2) ;
count_allele6q_b3_mat2=sum(allele6q_b3_mat2) ;
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count_allele7q_b3_mat2=sum(allele7q_b3_mat2) ;
count_allele8q_b3_mat2=sum(allele8q_b3_mat2) ;
count_allele9q_b3_mat2=sum(allele9q_b3_mat2) ;
count_allele10q_b3_mat2=sum(allele10q_b3_mat2) ;
count_allele11q_b3_mat2=sum(allele11q_b3_mat2) ;

freqallele1q_b3_mat2=sum(count_allele1q_b3_mat2)/(nobreeders3*2) ;
freqallele2q_b3_mat2=sum(count_allele2q_b3_mat2)/(nobreeders3*2) ;
freqallele3q_b3_mat2=sum(count_allele3q_b3_mat2)/(nobreeders3*2) ;
freqallele4q_b3_mat2=sum(count_allele4q_b3_mat2)/(nobreeders3*2) ;
freqallele5q_b3_mat2=sum(count_allele5q_b3_mat2)/(nobreeders3*2) ;
freqallele6q_b3_mat2=sum(count_allele6q_b3_mat2)/(nobreeders3*2) ;
freqallele7q_b3_mat2=sum(count_allele7q_b3_mat2)/(nobreeders3*2) ;
freqallele8q_b3_mat2=sum(count_allele8q_b3_mat2)/(nobreeders3*2) ;
freqallele9q_b3_mat2=sum(count_allele9q_b3_mat2)/(nobreeders3*2) ;
freqallele10q_b3_mat2=sum(count_allele10q_b3_mat2)/(nobreeders3*2) ;
freqallele11q_b3_mat2=sum(count_allele11q_b3_mat2)/(nobreeders3*2) ;

dist2=rand(totalrowsb2,2) ;

allele1q_b3_mat3=(dist2>0) & (dist2<freqallele1_G2) ;
allele2q_b3_mat3=(dist2>=freqallele1_G2) &
(dist2<(freqallele1_G2+freqallele2_G2)) ;
allele3q_b3_mat3=(dist2>=(freqallele1_G2+freqallele2_G2)) &
(dist2<(freqallele1_G2+freqallele2_G2+freqallele3_G2)) ;
allele4q_b3_mat3=(dist2>=(freqallele1_G2+freqallele2_G2+freqallele3_G2)) &
(dist2<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2)) ;
allele5q_b3_mat3=(dist2>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2)) &
(dist2<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2)) ;
allele6q_b3_mat3=(dist2>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2)) &
(dist2<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2)) ;
allele7q_b3_mat3=(dist2>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2+freqallele6_G2)) &
(dist2<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2)) ;
allele8q_b3_mat3=(dist2>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2)) &
(dist2<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2)) ;
allele9q_b3_mat3=(dist2>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2))
&
(dist2<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+freqallele9_G2)) ;
allele10q_b3_mat3=(dist2>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fr
eqallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+f
reqallele9_G2)) &
(dist2<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+freqallele9_G2+freqa
llele10_G2)) ;
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```
allele11q_b3_mat3=(dist2>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fr
eqallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+f
reqallele9_G2+freqallele10_G2)) & (dist2<1) ;

allele1q_b3_mat3(all(allele1q_b3_mat3==1,2),:)=[] ;
allele2q_b3_mat3(all(allele2q_b3_mat3==1,2),:)=[] ;
allele3q_b3_mat3(all(allele3q_b3_mat3==1,2),:)=[] ;
allele4q_b3_mat3(all(allele4q_b3_mat3==1,2),:)=[] ;
allele5q_b3_mat3(all(allele5q_b3_mat3==1,2),:)=[] ;
allele6q_b3_mat3(all(allele6q_b3_mat3==1,2),:)=[] ;
allele7q_b3_mat3(all(allele7q_b3_mat3==1,2),:)=[] ;
allele8q_b3_mat3(all(allele8q_b3_mat3==1,2),:)=[] ;
allele9q_b3_mat3(all(allele6q_b3_mat3==1,2),:)=[] ;
allele10q_b3_mat3(all(allele7q_b3_mat3==1,2),:)=[] ;
allele11q_b3_mat3(all(allele8q_b3_mat3==1,2),:)=[] ;

rows23=totalrowsb2-length(allele1q_b3_mat3(:,1));
rows24=totalrowsb2-length(allele2q_b3_mat3(:,1));
rows25=totalrowsb2-length(allele3q_b3_mat3(:,1)) ;
rows26=totalrowsb2-length(allele4q_b3_mat3(:,1));
rows27=totalrowsb2-length(allele5q_b3_mat3(:,1));
rows28=totalrowsb2-length(allele6q_b3_mat3(:,1));
rows29=totalrowsb2-length(allele7q_b3_mat3(:,1));
rows30=totalrowsb2-length(allele8q_b3_mat3(:,1));
rows31=totalrowsb2-length(allele9q_b3_mat3(:,1));
rows32=totalrowsb2-length(allele10q_b3_mat3(:,1));
rows33=totalrowsb2-length(allele11q_b3_mat3(:,1));

totalrowsb3=(rows23+rows24+rows25+rows26+rows27+rows28+rows29+rows30+rows3
1+rows32+rows33);

count_allele1q_b3_mat3=sum(allele1q_b3_mat3) ;
count_allele2q_b3_mat3=sum(allele2q_b3_mat3);
count_allele3q_b3_mat3=sum(allele3q_b3_mat3);
count_allele4q_b3_mat3=sum(allele4q_b3_mat3);
count_allele5q_b3_mat3=sum(allele5q_b3_mat3) ;
count_allele6q_b3_mat3=sum(allele6q_b3_mat3) ;
count_allele7q_b3_mat3=sum(allele7q_b3_mat3) ;
count_allele8q_b3_mat3=sum(allele8q_b3_mat3) ;
count_allele9q_b3_mat3=sum(allele9q_b3_mat3) ;
count_allele10q_b3_mat3=sum(allele10q_b3_mat3) ;
count_allele11q_b3_mat3=sum(allele11q_b3_mat3) ;

freqallele1q_b3_mat3=sum(count_allele1q_b3_mat3)/(nobreeders3*2) ;
freqallele2q_b3_mat3=sum(count_allele2q_b3_mat3)/(nobreeders3*2) ;
freqallele3q_b3_mat3=sum(count_allele3q_b3_mat3)/(nobreeders3*2) ;
freqallele4q_b3_mat3=sum(count_allele4q_b3_mat3)/(nobreeders3*2) ;
freqallele5q_b3_mat3=sum(count_allele5q_b3_mat3)/(nobreeders3*2) ;
freqallele6q_b3_mat3=sum(count_allele6q_b3_mat3)/(nobreeders3*2) ;
freqallele7q_b3_mat3=sum(count_allele7q_b3_mat3)/(nobreeders3*2) ;
freqallele8q_b3_mat3=sum(count_allele8q_b3_mat3)/(nobreeders3*2) ;
freqallele9q_b3_mat3=sum(count_allele9q_b3_mat3)/(nobreeders3*2) ;
freqallele10q_b3_mat3=sum(count_allele10q_b3_mat3)/(nobreeders3*2) ;
freqallele11q_b3_mat3=sum(count_allele11q_b3_mat3)/(nobreeders3*2) ;
```

```
dist3=rand(totalrowsb3,2) ;

allele1q_b3_mat4=(dist3>0) & (dist3<freqallele1_G2) ;
allele2q_b3_mat4=(dist3>=freqallele1_G2) &
(dist3<(freqallele1_G2+freqallele2_G2)) ;
allele3q_b3_mat4=(dist3>=(freqallele1_G2+freqallele2_G2)) &
(dist3<(freqallele1_G2+freqallele2_G2+freqallele3_G2)) ;
allele4q_b3_mat4=(dist3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2)) &
(dist3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2)) ;
allele5q_b3_mat4=(dist3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2)) &
(dist3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2)) ;
allele6q_b3_mat4=(dist3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2)) &
(dist3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2)) ;
allele7q_b3_mat4=(dist3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2+freqallele6_G2)) &
(dist3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2)) ;
allele8q_b3_mat4=(dist3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2)) &
(dist3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2)) ;
allele9q_b3_mat4=(dist3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fre
qallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2))
&
(dist3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+freqallele9_G2)) ;
allele10q_b3_mat4=(dist3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fr
eqallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+f
reqallele9_G2)) &
(dist3<(freqallele1_G2+freqallele2_G2+freqallele3_G2+freqallele4_G2+freqal
lele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+freqallele9_G2+freqa
llele10_G2)) ;
allele11q_b3_mat4=(dist3>=(freqallele1_G2+freqallele2_G2+freqallele3_G2+fr
eqallele4_G2+freqallele5_G2+freqallele6_G2+freqallele7_G2+freqallele8_G2+f
reqallele9_G2+freqallele10_G2)) & (dist3<1) ;

allele1q_b3_mat4(all(allele1q_b3_mat4==1,2),:)=[];
allele2q_b3_mat4(all(allele2q_b3_mat4==1,2),:)=[];
allele3q_b3_mat4(all(allele3q_b3_mat4==1,2),:)=[];
allele4q_b3_mat4(all(allele4q_b3_mat4==1,2),:)=[];
allele5q_b3_mat4(all(allele5q_b3_mat4==1,2),:)=[];
allele6q_b3_mat4(all(allele6q_b3_mat4==1,2),:)=[];
allele7q_b3_mat4(all(allele7q_b3_mat4==1,2),:)=[];
allele8q_b3_mat4(all(allele8q_b3_mat4==1,2),:)=[];
allele9q_b3_mat4(all(allele9q_b3_mat4==1,2),:)=[];
allele10q_b3_mat4(all(allele10q_b3_mat4==1,2),:)=[];
allele11q_b3_mat4(all(allele11q_b3_mat4==1,2),:)=[];

rows34=totalrowsb3-length(allele1q_b3_mat4(:,1)) ;
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rows35=totalrowsb3-length(allele2q_b3_mat4(:,1)) ;
rows36=totalrowsb3-length(allele3q_b3_mat4(:,1)) ;
rows37=totalrowsb3-length(allele4q_b3_mat4(:,1)) ;
rows38=totalrowsb3-length(allele5q_b3_mat4(:,1)) ;
rows39=totalrowsb3-length(allele6q_b3_mat4(:,1)) ;
rows40=totalrowsb3-length(allele7q_b3_mat4(:,1)) ;
rows41=totalrowsb3-length(allele8q_b3_mat4(:,1));
rows42=totalrowsb3-length(allele9q_b3_mat4(:,1)) ;
rows43=totalrowsb3-length(allele10q_b3_mat4(:,1)) ;
rows44=totalrowsb3-length(allele11q_b3_mat4(:,1));

totalrowsb4=(rows34+rows35+rows36+rows37+rows38+rows39+rows40+rows41+rows4
2+rows43+rows44);

count_allele1q_b3_mat4=sum(allele1q_b3_mat4) ;
count_allele2q_b3_mat4=sum(allele2q_b3_mat4) ;
count_allele3q_b3_mat4=sum(allele3q_b3_mat4) ;
count_allele4q_b3_mat4=sum(allele4q_b3_mat4) ;
count_allele5q_b3_mat4=sum(allele5q_b3_mat4) ;
count_allele6q_b3_mat4=sum(allele6q_b3_mat4) ;
count_allele7q_b3_mat4=sum(allele7q_b3_mat4) ;
count_allele8q_b3_mat4=sum(allele8q_b3_mat4) ;
count_allele9q_b3_mat4=sum(allele9q_b3_mat4) ;
count_allele10q_b3_mat4=sum(allele10q_b3_mat4) ;
count_allele11q_b3_mat4=sum(allele11q_b3_mat4) ;

freqallele1q_b3_mat4=sum(count_allele1q_b3_mat4)/(nobreeders3*2);
freqallele2q_b3_mat4=sum(count_allele2q_b3_mat4)/(nobreeders3*2);
freqallele3q_b3_mat4=sum(count_allele3q_b3_mat4)/(nobreeders3*2) ;
freqallele4q_b3_mat4=sum(count_allele4q_b3_mat4)/(nobreeders3*2) ;
freqallele5q_b3_mat4=sum(count_allele5q_b3_mat4)/(nobreeders3*2) ;
freqallele6q_b3_mat4=sum(count_allele6q_b3_mat4)/(nobreeders3*2) ;
freqallele7q_b3_mat4=sum(count_allele7q_b3_mat4)/(nobreeders3*2) ;
freqallele8q_b3_mat4=sum(count_allele8q_b3_mat4)/(nobreeders3*2) ;
freqallele9q_b3_mat4=sum(count_allele9q_b3_mat4)/(nobreeders3*2) ;
freqallele10q_b3_mat4=sum(count_allele10q_b3_mat4)/(nobreeders3*2) ;
freqallele11q_b3_mat4=sum(count_allele11q_b3_mat4)/(nobreeders3*2) ;

freqallele1q_b3=freqallele1q_b3_mat1+freqallele1q_b3_mat2+freqallele1q_b3_
mat3+freqallele1q_b3_mat4;
freqallele2q_b3=freqallele2q_b3_mat1+freqallele2q_b3_mat2+freqallele2q_b3_
mat3+freqallele2q_b3_mat4 ;
freqallele3q_b3=freqallele3q_b3_mat1+freqallele3q_b3_mat2+freqallele3q_b3_
mat3+freqallele3q_b3_mat4 ;
freqallele4q_b3=freqallele4q_b3_mat1+freqallele4q_b3_mat2+freqallele4q_b3_
mat3+freqallele4q_b3_mat4 ;
freqallele5q_b3=freqallele5q_b3_mat1+freqallele5q_b3_mat2+freqallele5q_b3_
mat3+freqallele6q_b3_mat4 ;
freqallele6q_b3=freqallele6q_b3_mat1+freqallele6q_b3_mat2+freqallele6q_b3_
mat3+freqallele7q_b3_mat4 ;
freqallele7q_b3=freqallele7q_b3_mat1+freqallele7q_b3_mat2+freqallele7q_b3_
mat3+freqallele7q_b3_mat4;
freqallele8q_b3=freqallele8q_b3_mat1+freqallele8q_b3_mat2+freqallele8q_b3_
mat3+freqallele8q_b3_mat4 ;
```

```
freqallele9q_b3=freqallele9q_b3_mat1+freqallele9q_b3_mat2+freqallele9q_b3_
mat3+freqallele9q_b3_mat4 ;
freqallele10q_b3=freqallele10q_b3_mat1+freqallele10q_b3_mat2+freqallele10q
_b3_mat3+freqallele10q_b3_mat4;
freqallele11q_b3=freqallele11q_b3_mat1+freqallele11q_b3_mat2+freqallele11q
_b3_mat3+freqallele11q_b3_mat4 ;


%Frequency of breeder contributions to next generation includes the drones
%they mate with

%These (this) queen genotype(s) had mated with (n) drones in last
generation
%Hence breeder contribution is

Ballelefreq1_G3=(2*freqallele1q_b3+freqallele1d_G2)/3;
Ballelefreq2_G3=(2*freqallele2q_b3+freqallele2d_G2)/3;
Ballelefreq3_G3=(2*freqallele3q_b3+freqallele3d_G2)/3;
Ballelefreq4_G3=(2*freqallele4q_b3+freqallele4d_G2)/3;
Ballelefreq5_G3=(2*freqallele5q_b3+freqallele5d_G2)/3;
Ballelefreq6_G3=(2*freqallele6q_b3+freqallele6d_G2)/3;
Ballelefreq7_G3=(2*freqallele7q_b3+freqallele7d_G2)/3;
Ballelefreq8_G3=(2*freqallele8q_b3+freqallele8d_G2)/3;
Ballelefreq9_G3=(2*freqallele9q_b3+freqallele9d_G2)/3;
Ballelefreq10_G3=(2*freqallele10q_b3+freqallele10d_G2)/3;
Ballelefreq11_G3=(2*freqallele11q_b3+freqallele11d_G2)/3;
%NOW USE ABOVE DISTRIBUTION TO GENERATE 100 QUEEN FREQUENY DIST. AND
%COMBINE WITH DRONE FREQUENCIES FROM BREEDERs 2 TO CREATE G3 DIST

DistGen3Q=rand(nqueens,2);

allele1q_G3_mat1=(DistGen3Q>0) & (DistGen3Q<Ballelefreq1_G3);
allele2q_G3_mat1=(DistGen3Q>=Ballelefreq1_G3) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3));
allele3q_G3_mat1=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3));
allele4q_G3_mat1=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3
_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3));
allele5q_G3_mat1=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3
_G3+Ballelefreq4_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3));
allele6q_G3_mat1=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3
_G3+Ballelefreq4_G3+Ballelefreq5_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3+Ballelefreq6_G3));
allele7q_G3_mat1=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3
_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3));
allele8q_G3_mat1=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3
_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3)) &
```

```
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3));
allele9q_G3_mat1=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3
_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballel
efreq8_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballelef
req9_G3));
allele10q_G3_mat1=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq
3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Balle
lefreq8_G3+Ballelefreq9_G3)) &
(DistGen3Q<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4_G
3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballelef
req9_G3+Ballelefreq10_G3));
allele11q_G3_mat1=(DistGen3Q>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq
3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Balle
lefreq8_G3+Ballelefreq9_G3+Ballelefreq10_G3)) & (DistGen3Q<1);

allele1q_G3_mat1(all(allele1q_G3_mat1==1,2),:)=[] ;
allele2q_G3_mat1(all(allele2q_G3_mat1==1,2),:)=[] ;
allele3q_G3_mat1(all(allele3q_G3_mat1==1,2),:)=[] ;
allele4q_G3_mat1(all(allele4q_G3_mat1==1,2),:)=[] ;
allele5q_G3_mat1(all(allele5q_G3_mat1==1,2),:)=[] ;
allele6q_G3_mat1(all(allele6q_G3_mat1==1,2),:)=[] ;
allele7q_G3_mat1(all(allele7q_G3_mat1==1,2),:)=[] ;
allele8q_G3_mat1(all(allele8q_G3_mat1==1,2),:)=[] ;
allele9q_G3_mat1(all(allele9q_G3_mat1==1,2),:)=[] ;
allele10q_G3_mat1(all(allele10q_G3_mat1==1,2),:)=[] ;
allele11q_G3_mat1(all(allele11q_G3_mat1==1,2),:)=[] ;

rows1=nqueens-length(allele1q_G3_mat1(:,1)) ;
rows2=nqueens-length(allele2q_G3_mat1(:,1)) ;
rows3=nqueens-length(allele3q_G3_mat1(:,1)) ;
rows4=nqueens-length(allele4q_G3_mat1(:,1)) ;
rows5=nqueens-length(allele5q_G3_mat1(:,1)) ;
rows6=nqueens-length(allele6q_G3_mat1(:,1)) ;
rows7=nqueens-length(allele7q_G3_mat1(:,1)) ;
rows8=nqueens-length(allele8q_G3_mat1(:,1))  ;
rows9=nqueens-length(allele9q_G3_mat1(:,1)) ;
rows10=nqueens-length(allele10q_G3_mat1(:,1)) ;
rows11=nqueens-length(allele11q_G3_mat1(:,1))  ;

totalrows=(rows1+rows2+rows3+rows4+rows5+rows6+rows7+rows8+rows9+rows10+ro
ws11);

count_allele1q_G3_mat1=sum(allele1q_G3_mat1) ;
count_allele2q_G3_mat1=sum(allele2q_G3_mat1) ;
count_allele3q_G3_mat1=sum(allele3q_G3_mat1) ;
count_allele4q_G3_mat1=sum(allele4q_G3_mat1) ;
count_allele5q_G3_mat1=sum(allele5q_G3_mat1) ;
count_allele6q_G3_mat1=sum(allele6q_G3_mat1) ;
count_allele7q_G3_mat1=sum(allele7q_G3_mat1) ;
count_allele8q_G3_mat1=sum(allele8q_G3_mat1) ;
count_allele9q_G3_mat1=sum(allele9q_G3_mat1) ;
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count_allele10q_G3_mat1=sum(allele10q_G3_mat1) ;
count_allele11q_G3_mat1=sum(allele11q_G3_mat1) ;

freqallele1q_G3_mat1=sum(count_allele1q_G3_mat1)/(nqueens*2) ;
freqallele2q_G3_mat1=sum(count_allele2q_G3_mat1)/(nqueens*2) ;
freqallele3q_G3_mat1=sum(count_allele3q_G3_mat1)/(nqueens*2) ;
freqallele4q_G3_mat1=sum(count_allele4q_G3_mat1)/(nqueens*2) ;
freqallele5q_G3_mat1=sum(count_allele5q_G3_mat1)/(nqueens*2) ;
freqallele6q_G3_mat1=sum(count_allele6q_G3_mat1)/(nqueens*2) ;
freqallele7q_G3_mat1=sum(count_allele7q_G3_mat1)/(nqueens*2) ;
freqallele8q_G3_mat1=sum(count_allele8q_G3_mat1)/(nqueens*2) ;
freqallele9q_G3_mat1=sum(count_allele9q_G3_mat1)/(nqueens*2) ;
freqallele10q_G3_mat1=sum(count_allele10q_G3_mat1)/(nqueens*2) ;
freqallele11q_G3_mat1=sum(count_allele11q_G3_mat1)/(nqueens*2) ;


DistGen3Q_2=rand(totalrows,2) ;

allele1q_G3_mat2=(DistGen3Q_2>0) & (DistGen3Q_2<Ballelefreq1_G3);
allele2q_G3_mat2=(DistGen3Q_2>=Ballelefreq1_G3) &
(DistGen3Q_2<(Ballelefreq1_G3+Ballelefreq2_G3));
allele3q_G3_mat2=(DistGen3Q_2>=(Ballelefreq1_G3+Ballelefreq2_G3)) &
(DistGen3Q_2<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3));
allele4q_G3_mat2=(DistGen3Q_2>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3)) &
(DistGen3Q_2<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3));
allele5q_G3_mat2=(DistGen3Q_2>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3)) &
(DistGen3Q_2<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3));
allele6q_G3_mat2=(DistGen3Q_2>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3)) &
(DistGen3Q_2<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3));
allele7q_G3_mat2=(DistGen3Q_2>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3)) &
(DistGen3Q_2<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3));
allele8q_G3_mat2=(DistGen3Q_2>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3)) &
(DistGen3Q_2<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3));
allele9q_G3_mat2=(DistGen3Q_2>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ball
elefreq8_G3)) &
(DistGen3Q_2<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballel
efreq9_G3));
allele10q_G3_mat2=(DistGen3Q_2>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefr
eq3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Bal
lelefreq8_G3+Ballelefreq9_G3)) &
(DistGen3Q_2<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
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_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballel
efreq9_G3+Ballelefreq10_G3));
allele11q_G3_mat2=(DistGen3Q_2>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefr
eq3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Bal
lelefreq8_G3+Ballelefreq9_G3+Ballelefreq10_G3)) & (DistGen3Q_2<1);

allele1q_G3_mat2(all(allele1q_G3_mat2==1,2),:)=[] ;
allele2q_G3_mat2(all(allele2q_G3_mat2==1,2),:)=[] ;
allele3q_G3_mat2(all(allele3q_G3_mat2==1,2),:)=[] ;
allele4q_G3_mat2(all(allele4q_G3_mat2==1,2),:)=[] ;
allele5q_G3_mat2(all(allele5q_G3_mat2==1,2),:)=[] ;
allele6q_G3_mat2(all(allele6q_G3_mat2==1,2),:)=[] ;
allele7q_G3_mat2(all(allele7q_G3_mat2==1,2),:)=[] ;
allele8q_G3_mat2(all(allele8q_G3_mat2==1,2),:)=[] ;
allele9q_G3_mat2(all(allele9q_G3_mat2==1,2),:)=[] ;
allele10q_G3_mat2(all(allele10q_G3_mat2==1,2),:)=[] ;
allele11q_G3_mat2(all(allele11q_G3_mat2==1,2),:)=[] ;

rows12=totalrows-length(allele1q_G3_mat2(:,1)) ;
rows13=totalrows-length(allele2q_G3_mat2(:,1)) ;
rows14=totalrows-length(allele3q_G3_mat2(:,1)) ;
rows15=totalrows-length(allele4q_G3_mat2(:,1)) ;
rows16=totalrows-length(allele5q_G3_mat2(:,1)) ;
rows17=totalrows-length(allele6q_G3_mat2(:,1)) ;
rows18=totalrows-length(allele7q_G3_mat2(:,1)) ;
rows19=totalrows-length(allele8q_G3_mat2(:,1))  ;
rows20=totalrows-length(allele9q_G3_mat2(:,1)) ;
rows21=totalrows-length(allele10q_G3_mat2(:,1)) ;
rows22=totalrows-length(allele11q_G3_mat2(:,1))  ;

totalrows2=(rows12+rows13+rows14+rows15+rows16+rows17+rows18+rows19+rows20
+rows21+rows22);

count_allele1q_G3_mat2=sum(allele1q_G3_mat2) ;
count_allele2q_G3_mat2=sum(allele2q_G3_mat2) ;
count_allele3q_G3_mat2=sum(allele3q_G3_mat2) ;
count_allele4q_G3_mat2=sum(allele4q_G3_mat2) ;
count_allele5q_G3_mat2=sum(allele5q_G3_mat2) ;
count_allele6q_G3_mat2=sum(allele6q_G3_mat2) ;
count_allele7q_G3_mat2=sum(allele7q_G3_mat2) ;
count_allele8q_G3_mat2=sum(allele8q_G3_mat2) ;
count_allele9q_G3_mat2=sum(allele9q_G3_mat2) ;
count_allele10q_G3_mat2=sum(allele10q_G3_mat2) ;
count_allele11q_G3_mat2=sum(allele11q_G3_mat2) ;

freqallele1q_G3_mat2=sum(count_allele1q_G3_mat2)/(nqueens*2) ;
freqallele2q_G3_mat2=sum(count_allele2q_G3_mat2)/(nqueens*2) ;
freqallele3q_G3_mat2=sum(count_allele3q_G3_mat2)/(nqueens*2) ;
freqallele4q_G3_mat2=sum(count_allele4q_G3_mat2)/(nqueens*2) ;
freqallele5q_G3_mat2=sum(count_allele5q_G3_mat2)/(nqueens*2) ;
freqallele6q_G3_mat2=sum(count_allele6q_G3_mat2)/(nqueens*2) ;
freqallele7q_G3_mat2=sum(count_allele7q_G3_mat2)/(nqueens*2) ;
freqallele8q_G3_mat2=sum(count_allele8q_G3_mat2)/(nqueens*2) ;
freqallele9q_G3_mat2=sum(count_allele9q_G3_mat2)/(nqueens*2) ;
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freqallele10q_G3_mat2=sum(count_allele10q_G3_mat2)/(nqueens*2) ;
freqallele11q_G3_mat2=sum(count_allele11q_G3_mat2)/(nqueens*2) ;

DistGen3Q_3=rand(totalrows2,2);

allele1q_G3_mat3=(DistGen3Q_3>0) & (DistGen3Q_3<Ballelefreq1_G3);
allele2q_G3_mat3=(DistGen3Q_3>=Ballelefreq1_G3) &
(DistGen3Q_3<(Ballelefreq1_G3+Ballelefreq2_G3));
allele3q_G3_mat3=(DistGen3Q_3>=(Ballelefreq1_G3+Ballelefreq2_G3)) &
(DistGen3Q_3<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3));
allele4q_G3_mat3=(DistGen3Q_3>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3)) &
(DistGen3Q_3<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3));
allele5q_G3_mat3=(DistGen3Q_3>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3)) &
(DistGen3Q_3<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3));
allele6q_G3_mat3=(DistGen3Q_3>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3)) &
(DistGen3Q_3<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3));
allele7q_G3_mat3=(DistGen3Q_3>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3)) &
(DistGen3Q_3<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3));
allele8q_G3_mat3=(DistGen3Q_3>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3)) &
(DistGen3Q_3<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3));
allele9q_G3_mat3=(DistGen3Q_3>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ball
elefreq8_G3)) &
(DistGen3Q_3<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballel
efreq9_G3));
allele10q_G3_mat3=(DistGen3Q_3>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefr
eq3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Bal
lelefreq8_G3+Ballelefreq9_G3)) &
(DistGen3Q_3<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballel
efreq9_G3+Ballelefreq10_G3));
allele11q_G3_mat3=(DistGen3Q_3>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefr
eq3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Bal
lelefreq8_G3+Ballelefreq9_G3+Ballelefreq10_G3)) & (DistGen3Q_3<1);

allele1q_G3_mat3(all(allele1q_G3_mat3==1,2),:)=[] ;
allele2q_G3_mat3(all(allele2q_G3_mat3==1,2),:)=[] ;
allele3q_G3_mat3(all(allele3q_G3_mat3==1,2),:)=[] ;
allele4q_G3_mat3(all(allele4q_G3_mat3==1,2),:)=[] ;
allele5q_G3_mat3(all(allele5q_G3_mat3==1,2),:)=[] ;
allele6q_G3_mat3(all(allele6q_G3_mat3==1,2),:)=[] ;
allele7q_G3_mat3(all(allele7q_G3_mat3==1,2),:)=[] ;
allele8q_G3_mat3(all(allele8q_G3_mat3==1,2),:)=[] ;
allele9q_G3_mat3(all(allele9q_G3_mat3==1,2),:)=[] ;
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allele10q_G3_mat3(all(allele10q_G3_mat3==1,2),:)=[] ;
allele11q_G3_mat3(all(allele11q_G3_mat3==1,2),:)=[] ;

rows23=totalrows2-length(allele1q_G3_mat3(:,1)) ;
rows24=totalrows2-length(allele2q_G3_mat3(:,1)) ;
rows25=totalrows2-length(allele3q_G3_mat3(:,1)) ;
rows26=totalrows2-length(allele4q_G3_mat3(:,1)) ;
rows27=totalrows2-length(allele5q_G3_mat3(:,1)) ;
rows28=totalrows2-length(allele6q_G3_mat3(:,1)) ;
rows29=totalrows2-length(allele7q_G3_mat3(:,1)) ;
rows30=totalrows2-length(allele8q_G3_mat3(:,1))  ;
rows31=totalrows2-length(allele9q_G3_mat3(:,1)) ;
rows32=totalrows2-length(allele10q_G3_mat3(:,1)) ;
rows33=totalrows2-length(allele11q_G3_mat3(:,1))  ;

totalrows3=(rows23+rows24+rows25+rows26+rows27+rows28+rows29+rows30+rows31
+rows32+rows33);

count_allele1q_G3_mat3=sum(allele1q_G3_mat3) ;
count_allele2q_G3_mat3=sum(allele2q_G3_mat3) ;
count_allele3q_G3_mat3=sum(allele3q_G3_mat3) ;
count_allele4q_G3_mat3=sum(allele4q_G3_mat3) ;
count_allele5q_G3_mat3=sum(allele5q_G3_mat3) ;
count_allele6q_G3_mat3=sum(allele6q_G3_mat3) ;
count_allele7q_G3_mat3=sum(allele7q_G3_mat3) ;
count_allele8q_G3_mat3=sum(allele8q_G3_mat3) ;
count_allele9q_G3_mat3=sum(allele9q_G3_mat3) ;
count_allele10q_G3_mat3=sum(allele10q_G3_mat3) ;
count_allele11q_G3_mat3=sum(allele11q_G3_mat3) ;

freqallele1q_G3_mat3=sum(count_allele1q_G3_mat3)/(nqueens*2) ;
freqallele2q_G3_mat3=sum(count_allele2q_G3_mat3)/(nqueens*2) ;
freqallele3q_G3_mat3=sum(count_allele3q_G3_mat3)/(nqueens*2) ;
freqallele4q_G3_mat3=sum(count_allele4q_G3_mat3)/(nqueens*2) ;
freqallele5q_G3_mat3=sum(count_allele5q_G3_mat3)/(nqueens*2) ;
freqallele6q_G3_mat3=sum(count_allele6q_G3_mat3)/(nqueens*2) ;
freqallele7q_G3_mat3=sum(count_allele7q_G3_mat3)/(nqueens*2) ;
freqallele8q_G3_mat3=sum(count_allele8q_G3_mat3)/(nqueens*2) ;
freqallele9q_G3_mat3=sum(count_allele9q_G3_mat3)/(nqueens*2) ;
freqallele10q_G3_mat3=sum(count_allele10q_G3_mat3)/(nqueens*2) ;
freqallele11q_G3_mat3=sum(count_allele11q_G3_mat3)/(nqueens*2) ;

DistGen3Q_4=rand(totalrows3,2) ;

allele1q_G3_mat4=(DistGen3Q_4>0) & (DistGen3Q_4<Ballelefreq1_G3);
allele2q_G3_mat4=(DistGen3Q_4>=Ballelefreq1_G3) &
(DistGen3Q_4<(Ballelefreq1_G3+Ballelefreq2_G3));
allele3q_G3_mat4=(DistGen3Q_4>=(Ballelefreq1_G3+Ballelefreq2_G3)) &
(DistGen3Q_4<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3));
allele4q_G3_mat4=(DistGen3Q_4>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3)) &
(DistGen3Q_4<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3));
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allele5q_G3_mat4=(DistGen3Q_4>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3)) &
(DistGen3Q_4<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3));
allele6q_G3_mat4=(DistGen3Q_4>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3)) &
(DistGen3Q_4<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3));
allele7q_G3_mat4=(DistGen3Q_4>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3)) &
(DistGen3Q_4<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3));
allele8q_G3_mat4=(DistGen3Q_4>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3)) &
(DistGen3Q_4<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3));
allele9q_G3_mat4=(DistGen3Q_4>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ball
elefreq8_G3)) &
(DistGen3Q_4<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballel
efreq9_G3));
allele10q_G3_mat4=(DistGen3Q_4>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefr
eq3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Bal
lelefreq8_G3+Ballelefreq9_G3)) &
(DistGen3Q_4<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballel
efreq9_G3+Ballelefreq10_G3));
allele11q_G3_mat4=(DistGen3Q_4>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefr
eq3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Bal
lelefreq8_G3+Ballelefreq9_G3+Ballelefreq10_G3)) & (DistGen3Q_4<1);

allele1q_G3_mat4(all(allele1q_G3_mat4==1,2),:)=[] ;
allele2q_G3_mat4(all(allele2q_G3_mat4==1,2),:)=[] ;
allele3q_G3_mat4(all(allele3q_G3_mat4==1,2),:)=[] ;
allele4q_G3_mat4(all(allele4q_G3_mat4==1,2),:)=[] ;
allele5q_G3_mat4(all(allele5q_G3_mat4==1,2),:)=[] ;
allele6q_G3_mat4(all(allele6q_G3_mat4==1,2),:)=[] ;
allele7q_G3_mat4(all(allele7q_G3_mat4==1,2),:)=[] ;
allele8q_G3_mat4(all(allele8q_G3_mat4==1,2),:)=[] ;
allele9q_G3_mat4(all(allele9q_G3_mat4==1,2),:)=[] ;
allele10q_G3_mat4(all(allele10q_G3_mat4==1,2),:)=[] ;
allele11q_G3_mat4(all(allele11q_G3_mat4==1,2),:)=[] ;

rows34=totalrows3-length(allele1q_G3_mat4(:,1)) ;
rows35=totalrows3-length(allele2q_G3_mat4(:,1)) ;
rows36=totalrows3-length(allele3q_G3_mat4(:,1)) ;
rows37=totalrows3-length(allele4q_G3_mat4(:,1)) ;
rows38=totalrows3-length(allele5q_G3_mat4(:,1)) ;
rows39=totalrows3-length(allele6q_G3_mat4(:,1)) ;
rows40=totalrows3-length(allele7q_G3_mat4(:,1)) ;
rows41=totalrows3-length(allele8q_G3_mat4(:,1)) ;
rows42=totalrows3-length(allele9q_G3_mat4(:,1)) ;
rows43=totalrows3-length(allele10q_G3_mat4(:,1)) ;
rows44=totalrows3-length(allele11q_G3_mat4(:,1)) ;
```

```
totalrows4=(rows34+rows35+rows36+rows37+rows38+rows39+rows40+rows41+rows42
+rows43+rows44);

count_allele1q_G3_mat4=sum(allele1q_G3_mat4) ;
count_allele2q_G3_mat4=sum(allele2q_G3_mat4) ;
count_allele3q_G3_mat4=sum(allele3q_G3_mat4) ;
count_allele4q_G3_mat4=sum(allele4q_G3_mat4) ;
count_allele5q_G3_mat4=sum(allele5q_G3_mat4) ;
count_allele6q_G3_mat4=sum(allele6q_G3_mat4) ;
count_allele7q_G3_mat4=sum(allele7q_G3_mat4) ;
count_allele8q_G3_mat4=sum(allele8q_G3_mat4) ;
count_allele9q_G3_mat4=sum(allele9q_G3_mat4) ;
count_allele10q_G3_mat4=sum(allele10q_G3_mat4) ;
count_allele11q_G3_mat4=sum(allele11q_G3_mat4) ;

freqallele1q_G3_mat4=sum(count_allele1q_G3_mat4)/(nqueens*2) ;
freqallele2q_G3_mat4=sum(count_allele2q_G3_mat4)/(nqueens*2) ;
freqallele3q_G3_mat4=sum(count_allele3q_G3_mat4)/(nqueens*2) ;
freqallele4q_G3_mat4=sum(count_allele4q_G3_mat4)/(nqueens*2) ;
freqallele5q_G3_mat4=sum(count_allele5q_G3_mat4)/(nqueens*2) ;
freqallele6q_G3_mat4=sum(count_allele6q_G3_mat4)/(nqueens*2) ;
freqallele7q_G3_mat4=sum(count_allele7q_G3_mat4)/(nqueens*2) ;
freqallele8q_G3_mat4=sum(count_allele8q_G3_mat4)/(nqueens*2) ;
freqallele9q_G3_mat4=sum(count_allele9q_G3_mat4)/(nqueens*2) ;
freqallele10q_G3_mat4=sum(count_allele10q_G3_mat4)/(nqueens*2) ;
freqallele11q_G3_mat4=sum(count_allele11q_G3_mat4)/(nqueens*2) ;

DistGen3Q_5=rand(totalrows4,2) ;

allele1q_G3_mat5=(DistGen3Q_5>0) & (DistGen3Q_5<Ballelefreq1_G3);
allele2q_G3_mat5=(DistGen3Q_5>=Ballelefreq1_G3) &
(DistGen3Q_5<(Ballelefreq1_G3+Ballelefreq2_G3));
allele3q_G3_mat5=(DistGen3Q_5>=(Ballelefreq1_G3+Ballelefreq2_G3)) &
(DistGen3Q_5<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3));
allele4q_G3_mat5=(DistGen3Q_5>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3)) &
(DistGen3Q_5<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3));
allele5q_G3_mat5=(DistGen3Q_5>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3)) &
(DistGen3Q_5<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3));
allele6q_G3_mat5=(DistGen3Q_5>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3)) &
(DistGen3Q_5<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3));
allele7q_G3_mat5=(DistGen3Q_5>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3)) &
(DistGen3Q_5<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3));
allele8q_G3_mat5=(DistGen3Q_5>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3)) &
```

```
(DistGen3Q_5<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3));
allele9q_G3_mat5=(DistGen3Q_5>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefre
q3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ball
elefreq8_G3)) &
(DistGen3Q_5<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballel
efreq9_G3));
allele10q_G3_mat5=(DistGen3Q_5>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefr
eq3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Bal
lelefreq8_G3+Ballelefreq9_G3)) &
(DistGen3Q_5<(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefreq3_G3+Ballelefreq4
_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Ballelefreq8_G3+Ballel
efreq9_G3+Ballelefreq10_G3));
allele11q_G3_mat5=(DistGen3Q_5>=(Ballelefreq1_G3+Ballelefreq2_G3+Ballelefr
eq3_G3+Ballelefreq4_G3+Ballelefreq5_G3+Ballelefreq6_G3+Ballelefreq7_G3+Bal
lelefreq8_G3+Ballelefreq9_G3+Ballelefreq10_G3)) & (DistGen3Q_5<1);

allele1q_G3_mat5(all(allele1q_G3_mat5==1,2),:)=[] ;
allele2q_G3_mat5(all(allele2q_G3_mat5==1,2),:)=[] ;
allele3q_G3_mat5(all(allele3q_G3_mat5==1,2),:)=[] ;
allele4q_G3_mat5(all(allele4q_G3_mat5==1,2),:)=[] ;
allele5q_G3_mat5(all(allele5q_G3_mat5==1,2),:)=[] ;
allele6q_G3_mat5(all(allele6q_G3_mat5==1,2),:)=[] ;
allele7q_G3_mat5(all(allele7q_G3_mat5==1,2),:)=[] ;
allele8q_G3_mat5(all(allele8q_G3_mat5==1,2),:)=[] ;
allele9q_G3_mat5(all(allele9q_G3_mat5==1,2),:)=[] ;
allele10q_G3_mat5(all(allele10q_G3_mat5==1,2),:)=[] ;
allele11q_G3_mat5(all(allele11q_G3_mat5==1,2),:)=[] ;

rows45=totalrows4-length(allele1q_G3_mat5(:,1)) ;
rows46=totalrows4-length(allele2q_G3_mat5(:,1)) ;
rows47=totalrows4-length(allele3q_G2_mat5(:,1)) ;
rows48=totalrows4-length(allele4q_G3_mat5(:,1)) ;
rows49=totalrows4-length(allele5q_G3_mat5(:,1)) ;
rows50=totalrows4-length(allele6q_G3_mat5(:,1)) ;
rows51=totalrows4-length(allele7q_G3_mat5(:,1)) ;
rows52=totalrows4-length(allele8q_G3_mat5(:,1)) ;
rows53=totalrows4-length(allele9q_G3_mat5(:,1)) ;
rows54=totalrows4-length(allele10q_G3_mat5(:,1)) ;
rows55=totalrows4-length(allele11q_G3_mat5(:,1)) ;

totalrows4=(rows45+rows46+rows47+rows48+rows49+rows50+rows51+rows52+rows53
+rows54+rows55);

count_allele1q_G3_mat5=sum(allele1q_G3_mat5) ;
count_allele2q_G3_mat5=sum(allele2q_G3_mat5) ;
count_allele3q_G3_mat5=sum(allele3q_G3_mat5) ;
count_allele4q_G3_mat5=sum(allele4q_G3_mat5) ;
count_allele5q_G3_mat5=sum(allele5q_G3_mat5) ;
count_allele6q_G3_mat5=sum(allele6q_G3_mat5) ;
count_allele7q_G3_mat5=sum(allele7q_G3_mat5) ;
count_allele8q_G3_mat5=sum(allele8q_G3_mat5) ;
count_allele9q_G3_mat5=sum(allele9q_G3_mat5) ;
```

```
count_allele10q_G3_mat5=sum(allele10q_G3_mat5) ;
count_allele11q_G3_mat5=sum(allele11q_G3_mat5) ;

freqallele1q_G3_mat5=sum(count_allele1q_G3_mat5)/(nqueens*2) ;
freqallele2q_G3_mat5=sum(count_allele2q_G3_mat5)/(nqueens*2) ;
freqallele3q_G3_mat5=sum(count_allele3q_G3_mat5)/(nqueens*2) ;
freqallele4q_G3_mat5=sum(count_allele4q_G3_mat5)/(nqueens*2) ;
freqallele5q_G3_mat5=sum(count_allele5q_G3_mat5)/(nqueens*2) ;
freqallele6q_G3_mat5=sum(count_allele6q_G3_mat5)/(nqueens*2) ;
freqallele7q_G3_mat5=sum(count_allele7q_G3_mat5)/(nqueens*2) ;
freqallele8q_G3_mat5=sum(count_allele8q_G3_mat5)/(nqueens*2) ;
freqallele9q_G3_mat5=sum(count_allele9q_G3_mat5)/(nqueens*2) ;
freqallele10q_G3_mat5=sum(count_allele10q_G3_mat5)/(nqueens*2) ;
freqallele11q_G3_mat5=sum(count_allele11q_G3_mat5)/(nqueens*2) ;

freqallele1q_G3=freqallele1q_G3_mat1+freqallele1q_G3_mat2+freqallele1q_G3_
mat3+freqallele1q_G3_mat4+freqallele1q_G3_mat5;
freqallele2q_G3=freqallele2q_G3_mat1+freqallele2q_G3_mat2+freqallele2q_G3_
mat3+freqallele2q_G3_mat4+freqallele2q_G3_mat5;
freqallele3q_G3=freqallele3q_G3_mat1+freqallele3q_G3_mat2+freqallele3q_G3_
mat3+freqallele3q_G3_mat4+freqallele3q_G3_mat5;
freqallele4q_G3=freqallele4q_G3_mat1+freqallele4q_G3_mat2+freqallele4q_G3_
mat3+freqallele4q_G3_mat4+freqallele4q_G3_mat5;
freqallele5q_G3=freqallele5q_G3_mat1+freqallele5q_G3_mat2+freqallele5q_G3_
mat3+freqallele5q_G3_mat4+freqallele5q_G3_mat5;
freqallele6q_G3=freqallele6q_G3_mat1+freqallele6q_G3_mat2+freqallele6q_G3_
mat3+freqallele6q_G3_mat4+freqallele6q_G3_mat5;
freqallele7q_G3=freqallele7q_G3_mat1+freqallele7q_G3_mat2+freqallele7q_G3_
mat3+freqallele7q_G3_mat4+freqallele7q_G3_mat5;
freqallele8q_G3=freqallele8q_G3_mat1+freqallele8q_G3_mat2+freqallele8q_G3_
mat3+freqallele8q_G3_mat4+freqallele8q_G3_mat5;
freqallele9q_G3=freqallele9q_G3_mat1+freqallele9q_G3_mat2+freqallele9q_G3_
mat3+freqallele9q_G3_mat4+freqallele9q_G3_mat5;
freqallele10q_G3=freqallele10q_G3_mat1+freqallele10q_G3_mat2+freqallele10q
_G3_mat3+freqallele10q_G3_mat4+freqallele10q_G3_mat5;
freqallele11q_G3=freqallele11q_G3_mat1+freqallele11q_G3_mat2+freqallele11q
_G3_mat3+freqallele11q_G3_mat4+freqallele11q_G3_mat5;


freqallele1_G3=(2*freqallele1q_G3+freqallele1d_G2)/3
freqallele2_G3=(2*freqallele2q_G3+freqallele2d_G2)/3
freqallele3_G3=(2*freqallele3q_G3+freqallele3d_G2)/3
freqallele4_G3=(2*freqallele4q_G3+freqallele4d_G2)/3
freqallele5_G3=(2*freqallele5q_G3+freqallele5d_G2)/3
freqallele6_G3=(2*freqallele6q_G3+freqallele6d_G2)/3
freqallele7_G3=(2*freqallele7q_G3+freqallele7d_G2)/3
freqallele8_G3=(2*freqallele8q_G3+freqallele8d_G2)/3
freqallele9_G3=(2*freqallele9q_G3+freqallele9d_G2)/3
freqallele10_G3=(2*freqallele10q_G3+freqallele10d_G2)/3
freqallele11_G3=(2*freqallele11q_G3+freqallele11d_G2)/3

mat1_G3(freqallele1)=freqallele1_G3
mat2_G3(freqallele1)=freqallele2_G3
mat3_G3(freqallele1)=freqallele3_G3
```

```
mat4_G3(freqallele1)=freqallele4_G3
mat5_G3(freqallele1)=freqallele5_G3
mat6_G3(freqallele1)=freqallele6_G3
mat7_G3(freqallele1)=freqallele7_G3
mat8_G3(freqallele1)=freqallele8_G3
mat9_G3(freqallele1)=freqallele9_G3
mat10_G3(freqallele1)=freqallele10_G3
mat11_G3(freqallele1)=freqallele11_G3

end


Matallele1=[mat1_G0; mat1_G1; mat1_G2; mat1_G3]
Matallele2=[mat2_G0; mat2_G1; mat2_G2; mat2_G3]
Matallele3=[mat3_G0; mat3_G1; mat3_G2; mat3_G3]
Matallele4=[mat4_G0; mat4_G1; mat4_G2; mat4_G3]
Matallele5=[mat5_G0; mat5_G1; mat5_G2; mat5_G3]
Matallele6=[mat6_G0; mat6_G1; mat6_G2; mat6_G3]
Matallele7=[mat7_G0; mat7_G1; mat7_G2; mat7_G3]
Matallele8=[mat8_G0; mat8_G1; mat8_G2; mat8_G3]
Matallele9=[mat9_G0; mat9_G1; mat9_G2; mat9_G3]
Matallele10=[mat10_G0; mat10_G1; mat10_G2; mat10_G3]
Matallele11=[mat11_G0; mat11_G1; mat11_G2; mat11_G3]

Alldata=[Matallele1; Matallele2; Matallele3; Matallele4; Matallele5;
Matallele6; Matallele7; Matallele8; Matallele9; Matallele10; Matallele11]
```

Appendix v - Denaturing Gel Gradient Electrophoresis (DGGE) methods

V.1 PCR protocol

The 30 µl mastermix for DGGE contained 2.5 mM $MgCl_2$, 200 µM each dNTP, 10 nM of each primer genoRfw-GC (5'CGC CCG CCG CGC CCC GCG GAC RAT ATG AAA AAT TAC ACA ATG A-3') and conscsdrev 5'-(TCA TCT CAT WTT TCA TTA TTC AAT-3') reactions with 6 µl 5X Colorless GoTaq® Reaction Buffer, and 1 U GoTaq® DNA Polymerase.

V.2 Touchdown PCR

Best results were obtained using the following protocol although non-specific products were amplified (Fig AV-1). An initial denaturing step of 94° C for 5min, was followed by one cycle at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72° for 1 min. For each subsequent cycle, a touchdown profile dropped the annealing temperature by 0.5°C per cycle to 47°C. This was followed by 20 cycles of 95°C for 1 min, annealing at 47°C for 1 min and extension at 72°C for 1 min. After a final 10 min cycle at 72°C, the temperature was dropped by 1°C (-1°C) every 2 min for 20 cycles, then incubated at 4°C.

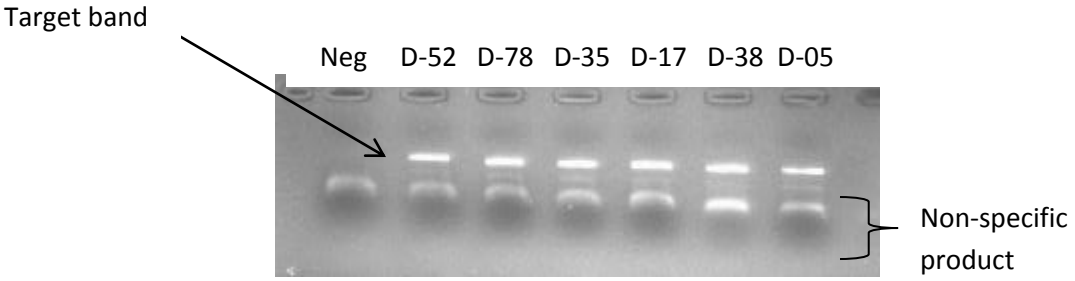

Fig AV-1. Non specific primimg and possible heterodimer products was an issue during PCR. I adjusted the PCR profile (increased annealing temp on touchdown protocol) to try and improve on product specificity with little success. I ran out this product type on a DGGE gel (see image below)

V.3 Running the DGGE gel

DGGE was conducted following the same protocol of Muyzer et al. (1993) using an Ingeny PhorU Electrophoresis System (Ingeny, Goes, Netherlands). DGGE gels contained 6% (w/v) polyacrylamide denaturing gradient gels with linear gradients from 15% to 55% of denaturing agent (where 100% is 7M urea and 40% (v/v) formamide). Base on product intensity on 1% agarose gel, 10-50 ng product was loaded onto the DGGE gel and run in 1 x TAE at 100V, 200ma at 60°C for 16 hours. Gels were stained with 1X SYBR-Gold (Invitrogen, Carlsbad) solution in 1X TAE buffer (Fig. Av-2)

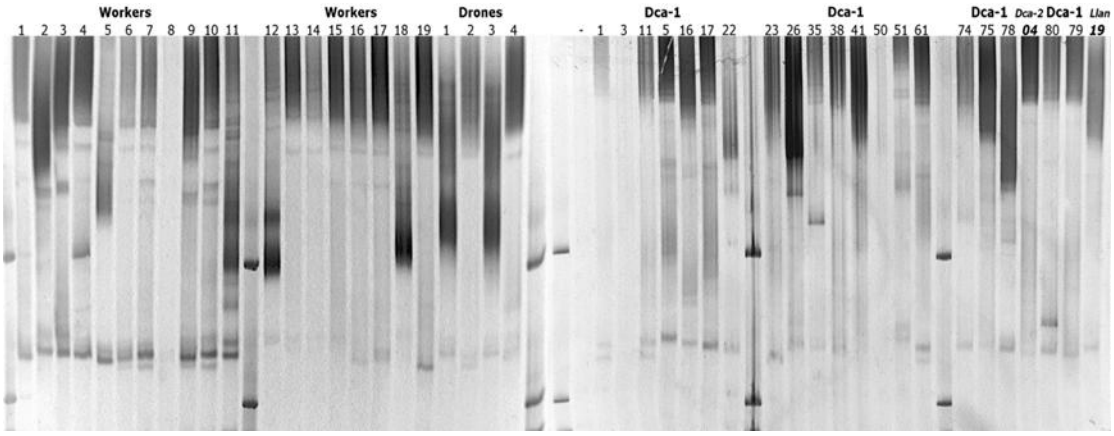

Fig Av-2. Image of DGGE gel using product amplified using the described above. Individual signatures were observed, but target fragments could not be isolated.

An effort was then made to clean up the product by excising the desired band on a low melt agarose gel (Sigma). Unfortunately, product yields were too low to perform DGGE (Fig. AV-3)

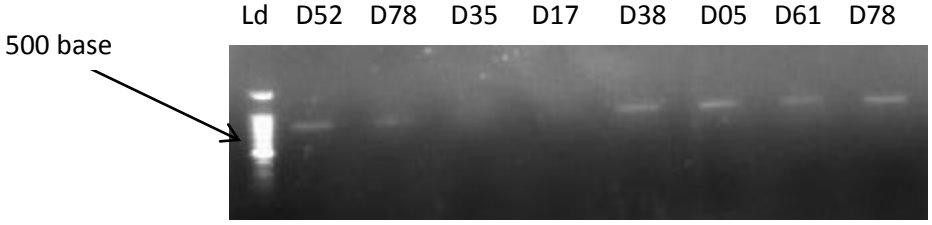

Fig. Av-3. Figure illustrates low post excision DNA yields