Bangor University

DOCTOR OF PHILOSOPHY

Population genetics and divergence in the Lesser Antillean anole (Anolis roquet)

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Award date:
2008

Awarding institution:
Bangor University

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POPULATION GENETICS AND DIVERGENCE IN THE LESSER ANTILLEAN ANOLE *Anolis roquet*

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A thesis submitted to Bangor University
in candidature for the degree of
Philosophiae Doctor

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U.K.
2008

ABSTRACT

The forces that drive population divergence and speciation in sexually reproducing organisms have long been debated with contributions from both theoretical and empirical data. Studies of genetic and phenotypic characters of previously isolated populations that meet in secondary contact provide one method of investigating these forces. In this study, multivariate techniques were used to analyse quantitative trait variation and dewlap hue variation in *Anolis roquet* along two geographically proximate and parallel transect in different habitats on the island of Martinique. Diagnostic PCR-RFLP was used to assign individuals to lineage. Nuclear DNA microsatellites were developed and employed for population genetic screening along the transects, and for the study of sex-biased dispersal. Microsatellite data were analysed using traditional summary statistics and Bayesian assignment analysis. These methods were further complemented with the modeling of diagnostic mtDNA markers, nuclear microsatellite markers, quantitative trait variation and dewlap hue variation with cline fitting techniques.

Results from quantitative traits, dewlap hue data and microsatellite data suggested that barriers to gene flow in accordance with an allopatric model of speciation existed on a coastal transect, and that these barriers were maintained by a balance between selection and dispersal. Moreover, potential for assortative mating and selective advantage of dewlap hue were determined for the coastal transect. In contrast, quantitative traits, dewlap hue data and microsatellite data suggested that there were no barriers to gene flow on a transect in transitional forest habitat, in spite of the close proximity of the transects and their shared geological history. Patterns of geographic variation on these two transects, together with environmental data, suggest that selection regimes on either side of the secondary contact zone in the transitional forest may be more convergent, while those either side of the secondary contact zone on the coast are more divergent. Secondary contact dynamics between previously isolated populations of *A. roquet* are therefore highly contingent on local natural selection pressure, and allopatry may play a role in the diversification of *Anolis* lineages, subject to selection pressures.

Sex-biased dispersal is common in sexually reproducing species, and has been widely documented in mammals and birds. Male-biased dispersal was tested in *A. roquet* using microsatellite data, and found to be highly significant. This finding was in accordance with predictions from the polygynous mating system of *A. roquet*, and agrees with similar results from an other species of anole. The finding of male-biased dispersal also goes some way to explaining the contrasting geographical patterns of maternal and biparental markers observed in *A. roquet*.

The implications of this work is discussed in relation to diversification of Lesser and Greater Antillean anoles, secondary contact zones of similar organisms and current views on the mechanism of speciation.

PREFACE

This thesis investigates population genetics and divergence in the Caribbean lizard *Anolis roquet*. The thesis is divided into three general themes; development of microsatellite markers (Chapter 3), sex-biased dispersal (Chapter 4) and secondary contact dynamics of *A. roquet* in northeastern Martinique (Chapters 5 and 6). These chapters are placed between General Introduction (Chapter 1), General Methods (Chapter 2) and General Discussion (Chapter 7) which place the work in a wider context.

The following publications were produced and in preparation during the course of the Ph.D:

1. Gow JL, Johansson H, Surget-Groba Y, Thorpe RS (2006). Ten polymorphic tetranucleotide microsatellite markers isolated from the *Anolis roquet* series of Caribbean lizards. *Molecular Ecology Notes*, **6**, 873-876. pp. 123-126, Appendix D.

2. Johansson, H, Surget-Groba, Y, Thorpe, R.S. (2008) Development of microsatellite markers in the St Lucia anole, *Anolis luciae. Molecular Ecology Resources*, **8**, 1408-1410. pp. 127-129, Appendix D.

3. Thorpe RS, Surget-Groba Y, Johansson H (2008) The relative importance of ecology and geographic isolation for speciation in anoles. *Philosophical Transactions of the Royal Society of London, Series B*. **363**, 3071-3081. pp. 130-140, Appendix D.

4. Johansson H, Surget-Groba Y, Thorpe RS (2008) Microsatellite data show evidence for male-biased dispersal in the Caribbean lizard *Anolis roquet. Molecular Ecology*, **17**, 4425-4432. pp. 141-148 Appendix D, and Chapter 4

5. Johansson H, Surget-Groba Y, Thorpe RS (2008) The roles of allopatric divergence and natural selection in quantitative trait variation across a secondary contact zone in the lizard *Anolis roquet. Molecular Ecology*. **17**, 5146-5156. pp 149-159, Appendix D and Chapter 5.

6. Johansson H, Surget-Groba Y, Thorpe RS (In preparation) Contrasting dynamics of secondary contact zones of *Anolis roquet* in northeastern Martinique. Chapter 5.

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GENERAL INTRODUCTION

1.1 SPECIATION

Speciation is a central tenet of evolutionary biology, and is recognised as a complex process. Research on speciation is framed within the context of species definitions, or concepts. A number of species definitions have been proposed (see Coyne and Orr (2004) and references therein), each with their own set of necessary properties for delimiting species. One of the most commonly used species concepts is that of the biological species concept (BSC), according to which a species is defined as:

> "groups of interbreeding natural populations that are reproductively isolated from other such groups"

> (Mayr, 1969)

Following this concept, groups of populations consist of distinct species under two conditions: when genetic differences between groups stop them from sharing the same area, or when they can share the same area but their genetic differences make them unable to produce viable young. The advantages of this species concept over many others is that it provides a clear delimitation between species, in that they must show reproductive isolation to fulfil the criteria of separate species (Coyne and Orr, 2004). However, there are many instances when the BSC cannot be applied, for example species in strict allopatry, to hybridising species or to asexual species. Some more unified concepts of speciation have been proposed, where species are defined as independently evolving meta-population lineages, and biological processes that maintain and drive divergence are considered as contingent properties (rather than necessary properties) (Simpson, 1951; Mayden, 1997; de Quieroz, 1998; Sites and Marshall, 2004; de Quieroz, 2005). Hence, species are mainly identified through the attributes that an evolving lineage gain with time. In this case, reproductive isolation becomes a contingent property with which to describe maintenance or divergence, similar to co-ancestry or ecological isolation. This is useful when studying processes of speciation, because at some stages of the process species status will be irresolvable (according to any concept), since accumulation of population differences may be concurrent. In this thesis, the focus is on processes that lead to speciation, and not on delineating species boundaries.

1.1.1 CLASSIC BIOGEOGRAPHIC PATTERNS OF SPECIATION

Biogeographical patterns of speciation were formally introduced by Darwin in the 'Origin of the Species', (1859) and their relative importance in speciation have remained under intense debate ever since (Mayr, 1969; Bush, 1975; Mayr, 1982; Gavrilets, 2005). The ultimate question for the consideration of biogeographical pattern is if geographical isolation (allopatric speciation, no gene flow) is a prerequisite for speciation to occur, or if speciation can in fact occur with low levels of gene flow (parapatric speciation), or with freely flowing genes (sympatric speciation) (Futuyama and Mayer, 1980). The biogeographical mode can limit the nature and strength of evolutionary forces that can potentially cause speciation, hence biogeographical patterns remain important in speciation studies (Turelli et al., 2001; Gavrilets, 2003; Rundle and Nosil, 2005).

ALLOPATRIC AND PARAPATRIC SPECIATION

Allopatric speciation occurs when geographically separated groups of species eventually evolve into independent species through genetic drift and natural selection. There is no genetic exchange between populations during the period of geographical isolation, and the initial barriers are physical. Allopatric speciation is further subdivided into vicariant and peripatric speciation. Peripatric speciation may occur when small populations become isolated, or when a few individuals colonises a new habitat. Island archipelagoes host some of the most famous examples of peripatric speciation, such as the adaptive radiation of the Hawaiian Drosophilids (Carson and Kaneshiro, 1976; Carson and Templeton, 1984).

Vicariant speciation is defined as populations splitting into two approximately equal sized populations. Evidence from nature for vicariant allopatric speciation is often inferred from concordance between species borders and geological features, for example: geminate species pairs such as shrimps in the genus of *Alpheus* separated by the Isthmus of Panama (Williams et al., 2001) and hybrid zones that coincide with geographical boundaries (Barton and Hewitt, 1985). The theory of allopatric speciation is uncontroversial and it has been shown theoretically that drift can lead to reproductive isolation, and that speciation through drift alone is a relatively slow process (Turelli et al., 2001; Gavrilets, 2003), that can be accelerated through natural selection.

Parapatric speciation falls midway between allopatric and sympatric speciation with regards to levels of gene flow (Slatkin, 1973; Haldane, 1948). Two main models of parap-

atric speciation are recognised: clinal and stepping stone models. In models of clinal speciation a species is distributed continuously in a varying environment, and subpopulations adapt to their local habitat in the presence of gene flow from other adjacent populations (Lande, 1982). Once subpopulations have differentiated there may be reinforcement. In the stepping stone model restricted gene flow between discrete populations theoretically facilitates for selection or drift to override gene flow and produces reproductive barriers as a by-product. Theoretical treatments suggest that speciation can occur by these processes, however under quite strict conditions of assortative mating (Felsenstein, 1981), specific combinations of mutation, selection and dispersal rates (Gavrilets et al., 2000), or small populations in combination with relatively high mutation rates (Gavrilets et al., 1998). Evidence for these models of speciation mainly come from studies of hybrid zones.

SYMPATRIC SPECIATION

Sympatric speciation, where reproductive isolation evolves where genes can be exchanged freely between populations, is the most controversial of the three biogeographical modes of speciation. Initial restriction of gene flow is caused by biological features of the organisms (Futuyama & Meyer 1980), not geography or distance. Sympatric speciation has to overcome two fundamental problems; antagonism between selection and recombination, and coexistence. Recent theoretical modelling tends to favour either disruptive natural (Smith, 1966; Felsenstein, 1981; Fry, 2003; Dieckmann and Doebeli, 1999; Kondashov and Kondrashov, 1999) or sexual selection (Turner and Burrows, 1995; Higashi et al., 1999; Takimoto et al., 2000; Arnegard and Kondrashov, 2004), to overcome these problems. The theory suggests that sympatric speciation is possible, however, it has been argued, only under very specific circumstances (Waxman and Gavrilets, 2005). There are only a few well-supported examples of sympatric speciation in nature. Potential examples include Arctic charr (*Salvelinus alpinus*) in Lake Galtabol, Iceland (Gislason et al., 1999), Tilapiine cichlids in caldera crater lakes in Cameroon (Schliewen et al., 1994) and cichlid fish in volcanic Nicaraguan lakes (Barluenga et al. 2006a; 2006b (but see Schliewen et al., 2006)).

1.1.2 ISOLATING BARRIERS

Within the different biogeographical models specific isolating barriers can act with varying strengths to drive speciation processes. Isolating barriers can be classified into those

that act before mating (e.g. ecological and behavioural) and those that act after mating (e.g. chromosome speciation, and genic incompatibilities). Some isolating barriers are conditional on certain aspects of a species' biology, for example polyploidy, which is common in plants, but not in animals, and only those that are considered relevant to diploid, sexually reproducing animals will be discussed here.

HABITAT ISOLATION

If spatial separation between population is based on biological differences and results in gene flow reduction, populations are considered to show habitat isolation. Ultimately, habitat isolation acts to reduce reproductive encounters between heterospecific populations, so that individuals from heterospecific populations mate less with each other than individuals from conspecific populations (Rundle and Nosil, 2005).

Habitat isolation, whether it evolves in allopatry, parapatry or sympatry, is due to natural selection. Evolution of habitat isolation in allopatry or parapatry is conceptually straightforward: two populations with limited or no geneflow adapt to their local habitat. If they attain sympatry, adaptation to local habitats is such that they show spatial isolation. Microspatial isolation occurs in leopard frogs *Rana blairi* and *R. pipiens* in Nevada, U.S.A. (Lynch, 1978), that are broadly sympatric in North America, but are separated by a preference for turbid, silty streams and clear sand bottom streams, respectively. Macrospatial isolation occurs in allopatrically distributed populations, in accordance with habitat type, of *Bombina bombina* and *B. variegata* near Cracow and Przemysl in Poland (Szymura and Barton, 1991). Occasionally both micro and macrospatial isolation coincide; two broadscale habitats may have a patchy border, where species distribution is in accordance with habitat. This occurs in a hybrid zone between the two closely relates species of crickets, *Gryllus firmus* and *G. pennsylvanicus* (Ross and Harrison, 2002), in which the mosaic structure of the hybrid zone reflects the underlying patchy distribution of sand and loam soil. Habitat isolation in the macrospatial form can act as a geographical barrier, hence in some environments only a slight difference can prevent gene flow and promote other isolating barriers. Importantly, if habitat isolation occurs in an allopatric model, the initial isolating barriers may be different from current barriers, and the relative importance of isolating barriers may be difficult to disentangle (Rundle and Nosil, 2005).

The theoretical models of speciation in sympatric scenarios involve disruptive selection for resource use (Dieckmann and Doebeli, 1999; Kondashov and Kondrashov, 1999) or disruptive selection on host use together with mating on the host (Rice, 1987), in which

habitat isolation can be an integral part of speciation. Of the potential examples of sympatric speciation mentioned above, speciation in fig wasps is thought to involve habitat isolation (Weiblen and Bush, 2002).

BEHAVIOURAL (ETHOLOGICAL) AND NON-ECOLOGICAL ISOLATION

Behavioural isolation includes all the differences between species that act to reduce the attraction (and consequently mating) between heterospecific individuals during the time when they are breeding. Similar to habitat isolation, behavioural isolation may arise as a consequence of natural selection.

Selection can act directly on mating preferences. In this case, female preference changes so that a particular male trait evolves in a particular direction, and alleles that alter preference confer a direct fitness benefit to the bearer (for example increasing fecundity) (Turelli et al., 2001). Alternatively, adaptive change can lead to female preference evolving for a by-product of adaptive change in one or several aspects of the sensory system. This process is known as sensory drive (or sensory exploitation) (Ryan, 1998; Proctor, 1991, 1992). Direct selection on preferences has the possibility to cause behavioural isolation, and ultimately reproductive isolation, provided that selection is acting in different directions in different populations. Proctor (1992) shows how evolution of sensory exploitation on trembling of males to attract females may have occurred in water mites (Acari: Parasitegona). A role for sensory drive in speciation of *Anolis* lizards has also been postulated (Leal and Fleishman, 2004), where dewlap colour provides the trait for female preference.

Selection can also act indirectly on mating preferences. Genes for female preference may not be the direct object of sexual selection. Instead, female preference evolves because certain genes become correlated with genes for specific male traits. These male traits are either more attractive to females, (also termed runaway sexual selection (Lande, 1981)) or they are an indication of higher male fitness, (also termed the good-genes model (Andersson, 1986). Theoretical modelling suggest that runaway selection and the good-genes model can cause divergent evolution under some circumstances; the trait or the preference may be altered by genetic drift or different mutations in different populations may affect evolution of traits or preferences. Divergent selection due to different environments may have a fitness-effect on alleles that affect either a preference or a trait (Lande, 1981; Pomiankowsky and Iwasa, 1998).

Selection on traits may be direct, where females may prefer males with locally adapted

traits. Invasion of new habitat can lead to either relaxed or altered selection, and this can either allow a trait to evolve past its previous optimum, or set off bouts of runaway selection (Irwin et al., 2001). Direct selection on traits can also involve intrasexual selection; members of one sex compete directly for access to resources attractive to the opposite sex, or for direct access to the opposite sex. It has been suggested that traits that increase armament, aggressive behaviour and territoriality are a consequence of intrasexual conflict, and if females can actively choose to mate with a male with a preferred trait, this type of selection may lead to a change in female preferences. It has been postulated that the prevalence of extra-pair copulations in many taxa, together with the observation that males often compete for resources and territories important to females, show evidence for intrasexual selection (Qvarnstrom and Forsgren, 1998).

Behavioural isolation need not involve sexually selected traits. Selection for species recognition may occur if natural selection promotes change in the same traits in both males and females. Divergent selection in different habitats could lead to behavioural isolation if both trait and preference change together in different populations. Examples from nature include Galapagos finches (*Geospiza scandens* and *G. fortis*) where behavioural differences are based mainly on differences in beak shape and body size, driven by adaptive divergence for resource use (Ratcliffe and Grant, 1983a,b) and the benthic and limnetic morphs of threespine stickleback, where variation in size, based on resource use, causes behavioural isolation (Schluter, 1993; Nagel and Schluter, 1998; Rundle, 2002).

Natural selection does not need to be involved in the evolution of behavioural isolation. Genetic drift on its own can theoretically cause this (Nei, 1983; Wu, 1985), however it would be a very slow process. Further, behavioural isolation can be due to cultural, gametic and mechanical isolation (Coyne and Orr, 2004). Elucidating which type of selection that ultimately causes behavioural isolation is usually difficult; natural selection can set off bouts of sexual selection, sexual selection can trigger other sexual selection, and several forces can act simultaneously.

POSTZYGOTIC BARRIERS

Postzygotic isolation, (i.e. barriers that act after fertilisation), can be either extrinsic or intrinsic. The extrinsic form is either ecological or behavioural. Hybrids may be inviable due to ecological selection (not adapted to habitat). The two morphs of North American threespine sticklebacks differ both ecologically and physiologically. Hybrids have

been shown to be viable in laboratory experiments but hybrids are rare in nature. In this case extrinsic postzygotic isolation is thought to be a direct result of adaptive evolution (Hatfield and Schluter, 1999).

Intrinsic postzygotic evolution is purely genetic, it consistently occurs in hybrids but never in pure species. Four kinds of genetic problems have been identified which can cause these hybrid difficulties: different ploidy levels (mainly plants), different chromosomal rearrangements, different alleles that do not function together in hybrids and infection by different endosymbionts (mainly arthropods).

Intrinsic postzygotic isolation is further divided into two classes, hybrid inviability and hybrid sterility. Hybrid inviability has been extensively studied in *Drosophila*, in laboratory settings (Coyne and Orr, 2004). Hybrid sterility takes two forms, behavioural or physiological (production of gametes fails). Behavioural intrinsic hybrid sterility occurs when viable gametes can be produced, but behavioural defects render, for example courtship behaviour, faulty. This is seen in close sister species of the butterfly genus *Anartia* where strong assortative mating for intraspecific mating exists, however viable hybrids are produced, and hybrids of certain crosses show reduced propensity to mate (Davies et al., 1997). The two anoles *A. aeneus* and *A. trinitatis* are an example of the physiological form. Hybrids of these two species can be produced, however the hybrids themselves cannot reproduce (Gorman et al., 1971).

Many species differ by chromosomal arrangements, including species of anoles in the Caribbean *Anolis* radiation (Creer et al. (2001) and references therein), and some types of arrangements, such as certain inversion and translocations can cause semi-sterility within species. The observation of chromosomal differences has led to the development of models of chromosomal speciation (reviewed in Rieseberg (2001)). The most recent modelling suggests that this type of speciation is difficult, because if a new arrangement (presumably semisterile) arises in a heterozygote form it should not be able to increase in frequency (Futuyama and Mayer, 1980). From nature chromosomal speciation in animals is rarely noted, and appears most likely if centric or monobrachial fusions are involved. Centric fusions occur when two apocentric chromosomes fuse to form one metacentric chromosome, whereas monobrachial fusion occurs when different centric fusions involve some but not all of the same chromosome. These type of fusions appear to be quite common in mammals (Lande, 1979; Nachman and Searle, 1995) and can cause problems in meiosis, but do not necessarily cause severe problems within species. One well-studied example of centric fusion occurs in the European house mouse (*Mus musculus*). A standard European house mouse has a diploid number of $2n = 40$ with only apocentric chro-

mosomes. However, there are at least 40 races of house mouse in Europe and Africa that have fewer chromosomes, which reflects the accumulation of centric fusions (Nachman and Searle, 1995; Hauffe and Searle, 1998; Pialek et al., 2001). Contact in hybrid zones between these races has shown that hybrids that are heterozygous for many centric fusions suffer significantly reduced fertility (Hauffe and Searle, 1998; Pialek et al., 2001).

The theory of genic incompatibilities (or genic speciation) attempts to show how alleles from different species cause intrinsic hybrid sterility or inviability. Between-locus incompatibilities, or the Dobzhansky-Muller model, describes (at its most simple) two allopatric populations evolving independently that experience many substitutions over long periods of time. Eventually, the populations become genetically distinct. It is probable that an evolved gene taken from one isolated population can function if placed in the genome of an another isolated population. However, it is unlikely that it would work better in a new genome, due to a build-up of genetic differences. Natural selection is not necessary for this process to take place; incompatibilities could simply be generated as geographically isolated species evolve (Orr, 2007). Single, or few, genetic incompatibilities need not have severe effects on the hybrids, but accumulation of many genetic compatibilities can have great effect. Incompatibilities may involve more than two genes, and in reality only one of the isolated species need to evolve to create genic incompatibilities (Coyne and Orr, 2004).

A classic example of postzygotic intrinsic barriers that follow a two-locus Dobzhansky-Muller model is the *Xiphophorus* fish. The platyfish (*X. maculatus*) are polymorphic for spots that are composed of macromelanophores, a close relative the swordtail (*X. helleri*) lacks these macromelanophores (Coyne and Orr, 2004). When these two are crossed all F_1 hybrids show an increased number of spots. Backcrossing F_1 hybrids to *X. helleri* results in half of the progeny lacking macromelanophores, whereas the other half develop phenotypes that range from forms similar to the F_1 phenotypes to invasive, often lethal, malignant melanomas. This occurs because spotted *X. maculatus* carry a sex-linked complex known as the *Tumor (Tu)* locus that specifies macromelanophores. The *Tu* locus is regulated by an autosomal suppressor locus. *Xiphophorus helleri* lacks the *Tu* locus and the suppressor. As a result, some of the backcross individuals between these species carries the *Tu* locus but not the suppressor locus and develop melanomas (Malitschek et al., 1995; Schartl, 1995; Meierjohann and Schartl, 2007).

1.1.3 REINFORCEMENT

Reinforcement is the enhancement of pre-zygotic isolation in sympatry, by natural selection. Fisher (1930) developed the first verbal model, followed by a population genetic model by Sawyer and Hartl (1981) and a quantitative model by Sved (1981a,b). However, many problems were noted with these models. First, selection-recombination antagonism means that very strong selection would be needed if there is gene flow between populations (Felsenstein, 1981). Second, reinforcement was shown to be likely only when populations already differed significantly in mating behaviour (pre-zygotic isolation) (Spencer et al., 1986). Third, too much gene flow was shown to be detrimental (swamping) (Servedio, 2000). Finally, reinforcement is self defeating, i.e. strength of selection for increased postzygotic isolation is proportional to the frequency of hybridisation, so any increase in prezygotic isolation reduces the strength of selection for further reinforcement (Spencer et al., 1986).

Eventually, a mathematical treatment by Liou and Price (1994) based on the model by Spencer et al. (1986), but explicitly taking sexual selection into account, showed that reinforcement could theoretically occur reasonably often, provided that hybrid fitness is low and populations already differ in female preference and male character. In their model two forces drive evolution of female preference; indirect selection in the form of a correlated response to sexual selection in males and direct selection due to the price paid for choosing a male of the wrong phenotype. Subsequently, it has been shown theoretically that reinforcement can occur under one and two way gene flow (Servedio, 1997), with assortative and preference mate choice (Servedio, 2000), with few or many hybrid incompatibilities (Kirkpatrick and Servedio, 1999) and regardless of the nature of selection (pre-versus postzygotic, intrinsic versus extrinsic) (Kirkpatrick, 2001; Servedio, 2001).

Most evidence from nature compares the strength of prezygotic isolation in sympatry versus allopatry. A recent example where reinforcement has been invoked to explain patterns of isolation is the walking stick insect (*Timema cristinae*). *Timema cristinae* show greater prezygotic isolation between populations that exchange migrants at medium rates. Reinforcement should not occur when there are negligible rates of gene flow (insufficient selection against hybrids) nor when there are high levels of gene flow (swamping) (Nosil et al., 2003). Frogs have proven to be good natural examples of reinforcement; male calls attract females and females tend to prefer conspecific calls; as occurs in the Australian treefrogs (*Hyla ewingi*) complex. Distant allopatric populations of *Hyla ewingi* and *H. verreauxi* have more similar calls than sympatric populations (Littlejohn, 1965). When given a choice experimentally, sympatric females from both species chose sympatric con-

specific males (Littlejohn and Loftus-Hills, 1968). Learning may also play a part in the evolution of reinforcement (Servedio et al., 2007).

1.1.4 STUDYING SPECIATION: OCEANIC ISLANDS, ADAPTIVE RADIATIONS AND HYBRID ZONES

The study of speciation in natural systems involves identifying initial and current isolating barriers that act on populations and estimating their relative contribution to lineage diversification.

A number of factors make oceanic island archipelagoes, such as Hawaii, the Galapagos islands, the Canaries and the Lesser Antilles ideal for evolutionary studies. The islands are isolated and the surrounding oceans impose clear boundaries which reduce gene flow between individual islands within archipelagos but also between islands and mainlands (Emerson, 2002). Oceanic islands are often small but in spite of their size they often contain a diversity of habitats and many endemic taxa. Many oceanic islands are geologically complex with historic and contemporary volcanic and erosional activity. The use of morphological data and molecular markers allows for reconstruction of phylogenies to test hypotheses of dispersal and diversification of lineages (Thorpe et al., 2004), evolution of species biology (Kambysellis et al., 1995), parallel evolution and the study of population level processes within and between island taxa (Brown et al., 1991), which can shed light on the importance of natural selection for shaping morphological variation (Malhotra, 1997), and the role of natural selection in speciation (Ogden et al., 2002; Thorpe et al., 2008).

Some island archipelagoes are almost synonymous with adaptive radiations, for example the *Drosophila* on Hawaii (Carson and Kaneshiro, 1976) and Darwin's finches on the Galapagos islands (Ratcliffe and Grant, 1983a,b). Adaptive radiation is the rapid diversification of one lineage into species that exploit a variety of different resources, and that differ in the morphological of physical trait used to exploit those resources (Schluter, 1996). Adaptive radiations are not restricted to oceanic archipelagoes. Cichlids (Kocher, 2004; Seehausen, 2006) and threespine sticklebacks (Schluter, 2000; McKinnon and Rundle, 2002), in the Great African lakes and North American postglacial lakes, respectively, are two of the best studied adaptive radiations. In particular, the role of ecological isolating barriers in divergence and ultimately speciation, have been extensively studied in adaptive radiations. Such work has contributed to the development of a theory of ecological speciation (Rundle and Nosil, 2005), or the process by which barriers to gene flow

evolve between populations as a result of ecologically-based divergent selection.

Hybrid zones are regions in which genetically distinct populations meet and produce hybrids. Similar to oceanic islands these areas have long been the subject of evolutionary research, and have been termed 'laboratories' for evolution (Barton and Hewitt, 1985; Hewitt, 2001). Populations that are genetically differentiated as a consequence of isolation may form clines in characters and alleles when they reach secondary contact, and the study of these clines may provide insights into the nature and strength of selective pressures that may be maintaining these clines (Barton and Hewitt, 1985; Szymura and Barton, 1991; Ross and Harrison, 2002; Leache and Cole, 2007). Volcanic activity and plate tectonics occasionally permit geographically isolated species on oceanic islands to come into secondary contact and form hybrid zones, and when this occurs an excellent opportunity to test the strength of allopatric speciation may be offered.

1.2 STUDY ISLANDS: THE LESSER ANTILLES AND MARTINIQUE

1.2.1 GEOGRAPHY AND GEOLOGY

The Lesser Antilles is an island chain in the Caribbean, approximately 850 km long, that extends from Sombrero in the north to Grenada in the South (Figure 1.1) (Martin-Kaye, 1969; Bouysse, 1983; Bouysse et al., 1983). The island arc lies on the margin of the Caribbean plate, where the Atlantic plate is thrust below it (Bouysse, 1983; Maury et al., 1990; Sigurdsson and Carey, 1991). The Lesser Antillean islands rests on a double arc; an inner, volcanically active arc (to the west) and an outer, inactive arc (to the east). The outer arc is thought to have been active from the Eocene to the Mid-oligocene, and the inner arc from the Miocene until the present (Sigurdsson and Carey, 1991). These arcs bifurcate north of Martinique. Southwest of Grenada lie the islands of Margarita, Los Testigos and Blanquilla which most likely form part of the double arc, however sediment buildup from South America obscures the exact relationship (Maury et al., 1990). Further to the west, the island of Bonaire sit on the Venezuelan Basin, and directly to the east of St Vincent is Barbados; neither of which are part of the Lesser Antilles double arc, but are relevant to the colonization by anole lizards of these islands.

Martinique is the largest of the islands in this chain, with an area of approximately 1116 km^2. Martinique is thought to consist of four precursor islands that surround a cen-
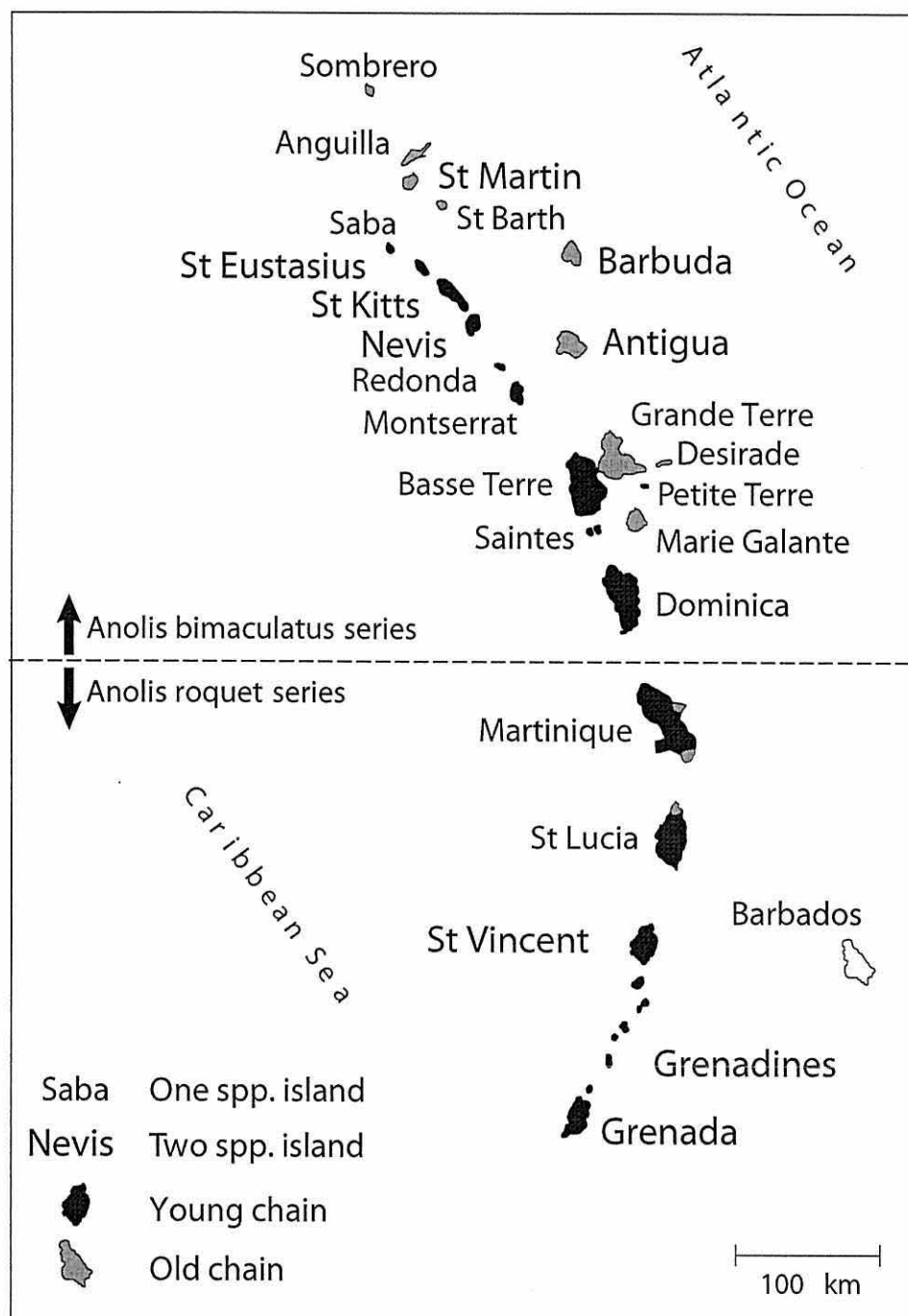
Figure 1.1: The Lesser Antilles with the *roquet* and *bimaculatus* series of anoles. Modified from (Thorpe et al., 2004), see text for details.

tral region. (Andreieff et al., 1976). The oldest precursors are the ancient regions of Caravelle and St Annes peninsula, part of the outer arc formed during the Eocene and early Miocene (9.5-19.5 Mya) (Andreieff et al., 1976; Sigurdsson and Carey, 1991). The younger (Miocene/Pleistocene) regions of Trois Ilets peninsula and a region in the north-

west (possibly precursor to Mt Conil) form the younger precursor islands (Andreieff et al., 1976; Sigurdsson and Carey, 1991). Volcanic activity and uplifting subsequently joined the precursor islands to what is now the island of Martinique (Andreieff et al., 1976; Maury et al., 1990; Sigurdsson and Carey, 1991). Episodic volcanic activity has migrated across the island; first from Morne Jacob towards Pitons du Carbet and back (4.4-2.4 Mya) (Bouysse et al., 1985) and more recently, Mt Pelee (0.5 Mya-present) (Traineau et al., 1989).

The highest peak on the island is Mt Pelee, which is one of the peaks in a massif including the Pitons Du Carbet and Morne Jacob in the northern part of the island. The eastern flanks of Mt Pelee slope relatively gently towards the coast, however the transition from mountain to coast on the western side is relatively precipitous.

1.2.2 CLIMATE AND VEGETATION

The normal climate of the low elevation islands in the Southern Lesser Antilles is warm tropical, with a mean temperature of 25-26°C at sea level, relative humidity around of 75%, and a prevalent north-easterly wind. Rainfall is showery and distributed over a drier season from January to May, and a wetter season from June to November (Beard, 1948). Substantial variation in rainfall occurs, with intermittent droughts, and the region is subjected to occasional hurricanes (Beard, 1948; Kimber, 1988). However, on the volcanic islands climatic variation occurs over very short distances. Hot air from the trade winds is deflected up steep mountain slopes to considerable altitudes, and is cooled by expansion. The moisture is condensed, forming clouds with subsequent heavy precipitation (Beard, 1948). Temperature, wind, sunshine and humidity are all affected in a similar manner.

Broad bioclimatic zones are recognised across the island. Beard (1948) combines the bioclimatic zones with vegetation data into four zones; dry belt climate, middle belt climate, mountain belt climate and upper mountain belt climate. These zones are further subdivided after vegetation type (Figure 1.3) (Beard, 1948; Lassere, 1979; Kimber, 1988). The transition from one bioclimatic zone to another is dependent on the combined effects of altitude, wind, precipitation and temperature.

Most coastal regions of Martinique lie in the dry belt climate; some areas, such as the Caravelle peninsula, and the northwestern coastal areas that lie in the rainshadow of Mt Pelee, are particularly dry. Exclusive to the east coast is the littoral woodland that includes heavily wind-stressed littoral hedge close to the sea, and the subsequent transitional stages

☰	Old arc (pre-Miocene)
▪	Intermediate arc (Miocene)
o	Morne Jacob volcanism (2.0-6.0 Mya)
⋮	Pitons du Carbet & Trois Ilets volcanism (3-0.5 Mya)
+	Mount Pelee volcanism (recent)
☐	Lamentin alluvial plain
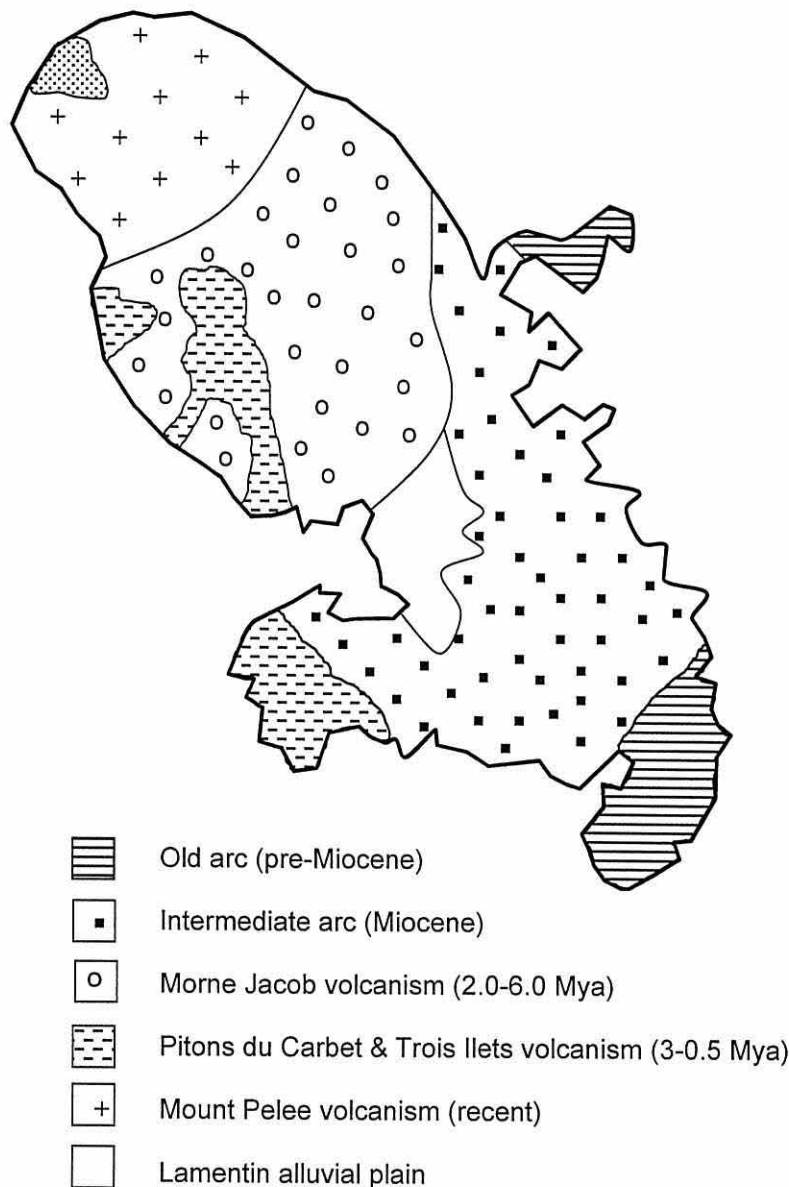
Figure 1.2: The geology of Martinique showing the main geological regions and their formation dates. After Bouysse (1983) (but see also Martin-Kaye (1969); Andreieff et al. (1976); Maury et al. (1990); Sigurdsson and Carey (1991)).

until relatively tall evergreen woodland succeed in the middle belt climate (Beard, 1948). Further inland the transition from littoral woodland to seasonal forest is evident as precipitation and sheltering from the wind increases further. Higher up on the slopes tropical rainforest emerges (mountain belt climate) and at the very peaks of the mountains there is elfin woodland (upper mountain belt climate). On the west coast the equivalent plant communities to the east coast littoral woodland are bush and shrubland, and lowland areas (excluding coastal regions) on Martinique are dominated by seasonal forests and bushland

(Kimber, 1988).
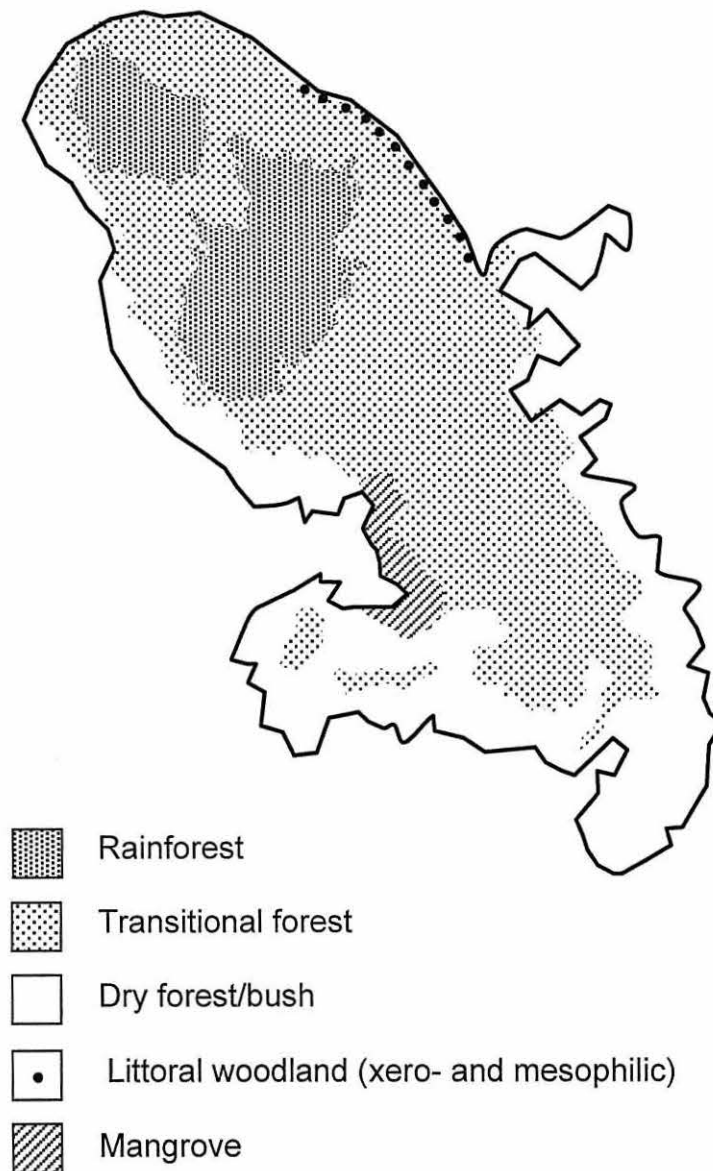
Figure 1.3: Bioclimatic and habitat map of Martinique. After Lassere (1979).

1.3 STUDY ANIMALS: *Anolis* LIZARDS AND *Anolis roquet*

1.3.1 *Anolis* LIZARDS

Anoles (Squamata: Iguanidae: Polychrotinae) are small, mainly insectivorous, primarily arboreal lizards found throughout the Caribbean, Central America, northern South America and the south-eastern U.S.A.. With more than 300 described species, *Anolis* is one of the most speciose amniote genera (Poe, 2004). The genus *Polychrus*, with five species present in central and south America, is *Anolis* closest relative (Frost et al., 2001).

There are at least 300 species of anole, and of these 154 species reside in the Caribbean (Nicholson et al., 2005). The majority (111 sp) reside in the Greater Antilles. Several lines of evidence suggest that the genus is very old: immunological estimates suggest an age of at least 40 million years (Shochat and Dessauer, 1981), *Anolis* fossilized in amber from the Miocene (20-23 Mya) was found in the Dominican Republic, suggesting that anoles had colonised the Caribbean by then (Rieppel, 1980), and molecular phylogenetic estimation suggests evolutionary divergence in the genus about 30 million years ago (Losos et al., 2006). Phylogenetic studies have shown that the genus originally colonised from South America and probably invaded the Caribbean twice (Jackman et al., 1999; Nicholson et al., 2005). Whilst one colonisation wave is believed to have given rise to the *roquet*-series of anoles, the other gave rise to all other anoles in the Caribbean (Thorpe et al., 2004). In comparison with the Greater Antillean islands where there are up to 55 endemic species of anole on single islands (Losos et al., 2003), the Lesser Antilles islands support depauperate lizard communities, with only one or two species per island (Gorman and Atkins, 1969; Thorpe and Stenson, 2003). The forty or so species inhabiting the Lesser Antillean islands divide into two series of *Anolis*; the *bimaculatus* and *roquet*-series (Figure 1.1) (Gorman and Atkins, 1969).

Two morphological features distinguish anoles from other iguanids; sub-digital toe pads with laterally expandable lamellae that are covered with modified scale derivatives, or setae (Irschick et al., 1996), and a dewlap (gular throat fan) (Guyer and Savage, 1986). The specialized toe pads allow these lizards to adhere to smooth surfaces, and pad size in different species is related to clinging ability (Irschick et al., 1996). The dewlap is present in most males and in some species also in the female. It is used for inter-species recognition and intra-species social interaction, and also to display pursuit-deterrent signals against predators (Leal, 1999). The pattern of head movement that accompanies dewlap extension is species specific in sympatric anoles (Williams and Rand, 1977; Macedonia et al.,
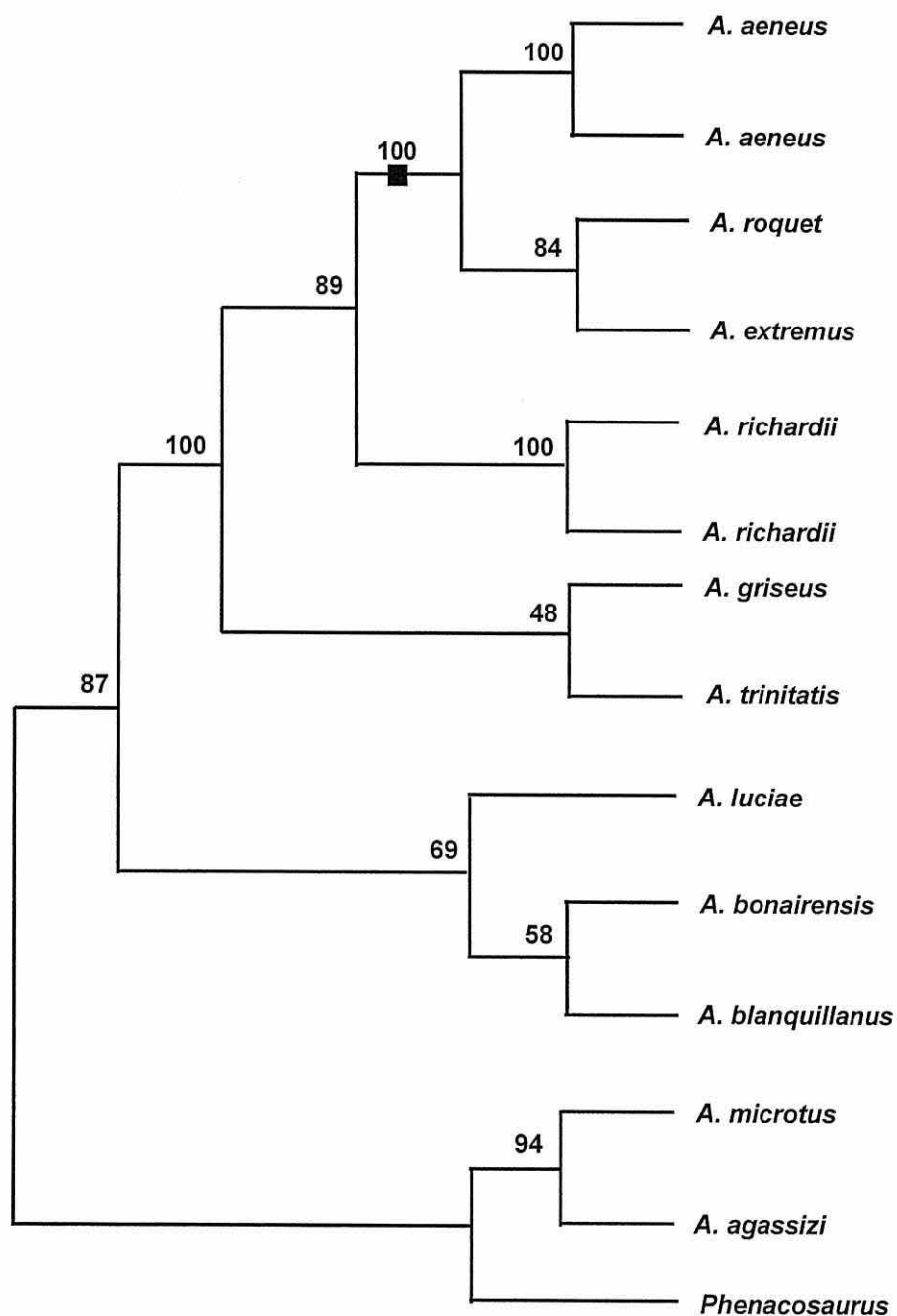
Figure 1.4: The most parsimonious tree for the *roquet*-series of anoles; mtDNA sequence data and allozyme data combined. Bootstrap values are above branches. Black box indicates the derived karyotype of 2n=34. After Creer et al. (2001). Figure 1.6 shows the *A. roquet/extremus* relationship, resolved more recently by (Thorpe and Stenson, 2003).

1994; Jenssen et al., 2000). In addition, even though dewlap colouration is often species specific it can show considerable intraspecific variation that is believed to be an adaptation for increased detectability in habitats (Williams and Rand, 1977; Thorpe, 2002; Leal

and Fleishman, 2004 (but see Macedonia, 2001; Macedonia et al., 2003)).

Various aspects of ecology and evolution have been studied in Caribbean island anole communities, reviewed in Losos (1994), Roughgarden (1995) and Thorpe et al. (2004) and they remain important organisms with which to test evolutionary and ecological hypotheses.

Figure 1.5: Morphological variation in *A .roquet*. The lizard on the left is typical of the NW lineage at Basse-Pointe. The lizard on the right is typical of the C lineage at Anse Charpentier. Both localities are situated on the Atlantic coast, less than 16 km apart. (Photographs: Yann Surget-Groba)

1.3.2 THE *roquet*-GROUP OF ANOLES AND *Anolis roquet*

The *roquet*-series of *anolis* lizards is found from Grenada and the Grenadines to Martinique in the Lesser Antilles, and Martinique is the northern-most island for the series distribution (Figure 1.1) (Gorman and Atkins, 1969; Creer et al., 2001; Thorpe and Stenson, 2003). Two islands (areas) support two sympatic species: Grenada and the Grenadines are inhabited by *A. aeneus* and *A richardii* and St Vincent by *A. trinitatis* and *A. griseus*. Members of the series are also found on Barbados to the east (*Anolis extremus*) and on the islands of Blanquilla (*A. blanquillanus*) and Bonaire (*A. bonairensis*) to the west of the Lesser Antilles island chain (Gorman and Atkins, 1969; Creer et al., 2001). Research on the evolution and phylogeny of Lesser Antillean anoles has been extensive. Osteological research provided the first systematic classification (Etheridge 1959, See Jackman et al. 1999), followed by karyological research (Gorman and Atkins, 1969) and subsequently allozyme analysis (Yang et al., 1974). This early research provided biogeographical and phylogenetic hypotheses which are to some extent still relevant. Creer et al. (2001) reanalysed allozyme data and combined this with mtDNA data to produce the most comprehensive phylogeny that exists for this group (Figure 1.4).

There are no known natural hybrids in the *roquet*-series, however many anoles, including *A. trinitatis* and *A. aeneus*, have experienced human-mediated invasion to other islands (Gorman et al., 1971). These two species are now sympatric on Trinidad and they have been found to hybridise and produce morphologically intermediate F_1 offspring that are sterile (Gorman et al., 1971). Anoles in the *roquet*-series hence appear to form good species.

Anolis roquet is endemic to Martinique, where it is found in abundance in most habitats; dry rain shadow to wet lowlands and high forests, pasture scrub, shrub trees, cacti, xeric woodlands, gardens, buildings and ruins, and coastal groves (Schwartz and Henderson, 1991). Similar to other Lesser Antillean anole lizards there is also considerable intra-island geographical variation in pattern and colour in *A. roquet* (Lazell, 1972; Thorpe and Stenson, 2003) (Figure. 1.5). The morphological variation prompted Lazell (1972) to designate six subspecies, however subsequent allozyme analysis of three of the six designated subspecies revealed low levels of variation between subspecies (Gorman and Kim, 1975). However, Gorman and Kim's (1975) sampling for the allozyme analysis was such that all lizards used (three in total) could have been from the central lineage (see below). Recently, Thorpe and Stenson (2003) showed that there are four main mtDNA lineages of *A. roquet* on Martinique (Figure 1.6), and that these lineages are closely associated with the precursor islands. Hence, independent *Anolis roquet* lineages are thought
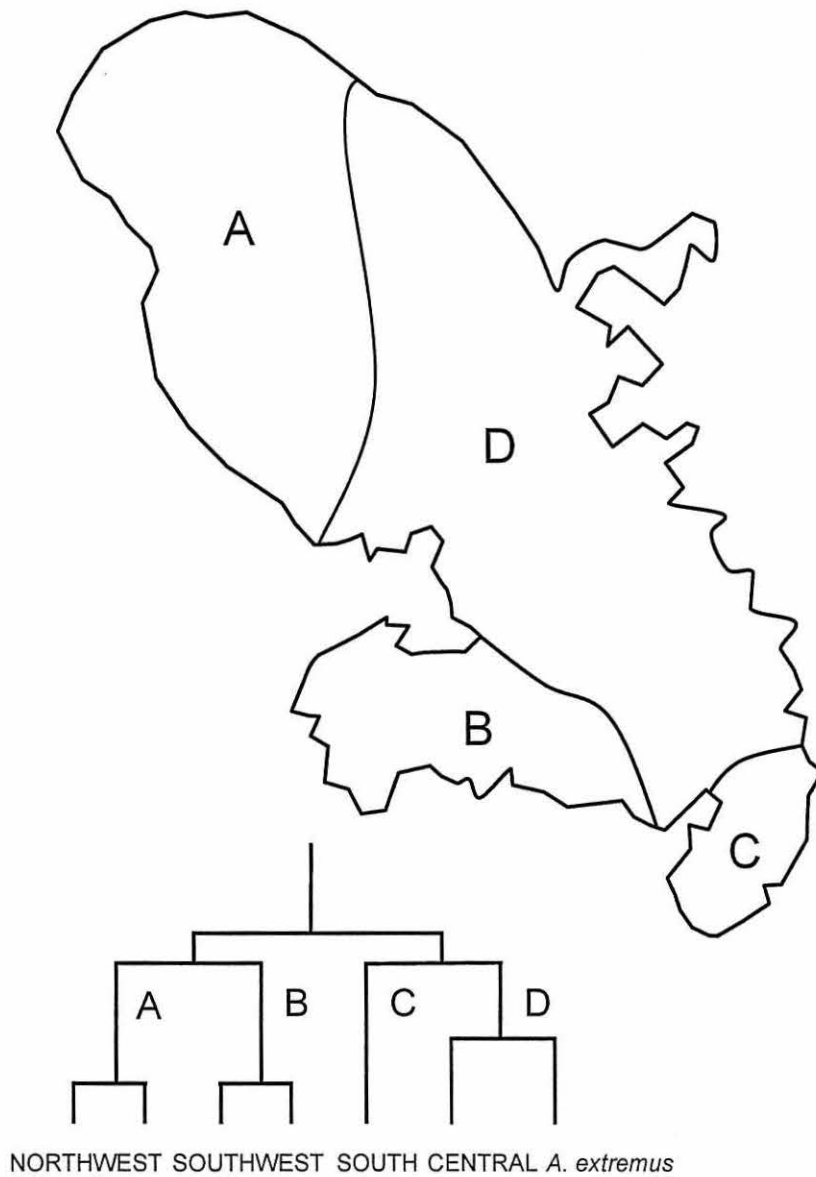
Figure 1.6: Phylogenetic lineage boundaries for *A. roquet* on Martinique. Adapted from Thorpe and Stenson (2003).

to have evolved on the precursor islands, and when the precursor islands were eventually connected to form present-day Martinique, lineages came into secondary contact. Three major contact zones have been identified on Martinique (Figure 1.6), where divergence between the different lineages has been estimated at between 5 and 8 million years, probably followed by 1-1.5 million years of secondary contact (Thorpe and Stenson, 2003; Thorpe et al., 2008). The Thorpe and Stenson (2003) study also revealed that *A. extremus* is nested within *A. roquet* as a sister clade to the central lineage, suggesting that Barbados was colonised by lizards from the central lineage (Figure 1.6).

Body dimensions and scalation are highly variable in numerous anole species, including *A. roquet*, and these traits typically correlate with rainfall and altitude (Thorpe et al., 2004). Overall colour and pattern variation appear to be a response to biotope and do not generally reflect lineages (Thorpe and Stenson, 2003). Focusing on one secondary contact zone, Ogden and Thorpe (2002) found no evidence that past allopatric divergence was reflected in current geneflow and quantitative trait variation. Instead, both geneflow and quantitative trait variation were found to be linked to sharp changes in ecotones, suggesting only ecological barriers to geneflow. Despite this general pattern, Thorpe and Stenson (2003) identified two areas where quantitative trait variation, at least superficially, seems to correlate to lineage, the first one in southern Martinique (Ste Anne peninsula) and the second on the north Atlantic coast (Figure 1.5).

The aim of this thesis was to investigate one area where a contact zone appeared to contradict the general pattern, northeastern Martinique. In addition to 15 quantitative traits characters that were used by Ogden and Thorpe (2002), another five traits were added (dewlap length, % black on head and cloak, chevron intensity and occipital A mark), and together with spectrophotometric measure of dewlap hue variation these traits were modelled across the secondary contact zones. Sample numbers were substantially increased from previous studies, for both genetic and morphometric analyses. More microsatellites were developed, and to complement the conventional methodology of Ogden and Thorpe (2002), the utility of cline fitting techniques and Bayesian assignment methods in these secondary contact zones was also investigated. During the course of the work, questions about the different geographic patterns in mtDNA and microsatellite data arose, and were investigated.

GENERAL METHODS

2.1   SAMPLING

Samples from previous sampling trips (Ogden and Thorpe, 2002; Thorpe and Stenson, 2003) were used for microsatellite development (Chapter 3), and for designing the diagnostic PCR-RFLP assay (Chapter 5). *Anolis roquet* tailtips were collected on Martinique between April and June in 2005, 2006 and 2007. For Chapter 4, 372 tail tips were collected from ten island-wide localities, and a further 281 tail tips were collected from seven localities along a short transect. For Chapters 5 and 6, a total of 765 tail tips were collected on two transects. Ten adult male lizards were also collected at each sampled locality to collect quantitative trait and hue data for Chapters 5 and 6 (see below). Lizards were released at the place of capture unharmed.

2.2   QUANTITATIVE TRAITS AND HUE

2.2.1   QUANTITATIVE TRAITS

The methods employed in Chapter 5 to measure body dimensions and count scales are a modified subset of those described by Malhotra (1992) and Ogden et al. (2002), and the determination of marking and colour patterns follow Thorpe and Stenson (2003). Ten adult males from each sampled locality were caught by hand or noose. In total, 20 characters were recorded from four different character sets: body dimensions, scale counts, markings and hue (Table 2.1 and Figure 2.1). Prior to recording body measurements and marking patterns, photographs were taken of the lizards using a Canon EOS 350D fitted with a 100 mm Canon macro lens and a Macro Twin Lite MT-24EX flash. The photographs were used to confirm scoring of marking patterns and to perform dorsal and ventral scale counts. Further, a fourth character set; a measure of hue based on the relative proportion of green, red and blue pixels within a standardised area on the dorsal trunk, was extracted from these photographs using Photoshop (Adobe Systems,U.S.A). Body dimension measurements were taken in millimetres using electronic digital callipers (Linear Tools, U.K.), accurate to two decimal points, and dorsal and ventral photographs taken (as above) for scale counts. Lizards were left to recover after measurement, and then returned unharmed to the place of capture.

Body dimensions were adjusted against snout-vent length by analysis of covariance (ANCOVA) to adjust for age-related effects. Categorical markings data and two scale counts (SSC and PSC) with non-normal distributions were entered into a principal components

analysis (PCA) to extract seven new components. Principal component analysis is an unconstrained ordination technique that can be used with highly correlated data to produce new uncorrelated indices that can be used to further analyse variation and pattern in datasets (Manly, 2000; McGarigal et al., 2000). Component scores, body dimensions, two raw scale count variables (DSC and VSC) and two hue variables (red and green) were combined in canonical variate analyses (CVA) using each transect locality as a group. Canonical variate analyses discriminates among pre-specified groups of sampling entities based on multivariate discriminating variables. Gradients of dominant, underlying variation in the dataset are maximised among the pre-defined groups and minimised within-groups (McGarigal et al., 2000). The individual scores (normalised so that the pooled within-group standard deviation is unity) can be plotted along the transect for a graphical representation of patterns of variation.

Table 2.1: Quantitative trait data collection

Category	Abbreviation	No. on figure	Trait
Body Dimensions	SVL	1	length from tip of snout to vent
	JL	2	length from tip of snout to end of jaw
	HL	3	length from tip of snout to just behind ear opening
	HD	4	depth of head just above the eyes
	HW	5	width of head just behind the eyes
	ULL	6	length from mid-abdomen to knee joint
	LLL	7	length from knee-joint to heel joint
	DLL	8	length of extended dewlap from chin to widest point
Scalation	DSC	9	number of dorsal scales from precisely before front-legs to precisely before back-leg
	VSC	10	number of central scales from precisely before the back-legs and including dewlap
	PMSC	11	number of postmental scales
	SSSC	12	number of scales between the supra-orbital semicircle
Markings	LPH	13	number of light patches on head
	LPA	14	number of light patches on anterior body
	LPB	15	number of light patches on posterior body
	CHV	16	number of dorsal chevrons
	CHVI	17	chevron intensity
	A	18	occipital A mark
	HEAD	20	% cover of black head (Categories: 0, 1-25, 26-50, 51-75, 76-100)
	CLOAK	19	% cover of black cloak (Categories as above)
Hue			proportion of red, green and blue pixels in a standard area just behind the front legs

2.2.2 HUE

For each of the ten adult males lizards collected for quantitative trait data, spectrophotometrical readings were taken from the anterior and posterior dewlap (Chapter 6). A 200 μ receptor fibre was held at 45° to the dewlap by a purpose-made, matte black attachment. At least three hue recordings per region, from near UV to the red spectrum, were taken as the diffuse reflectance from the surface, as a percentage of a WS-2 white standard. An AvaSpec-2048 spectrometer, with a AvaLight-XE xenon pulsed light source (Avantes, Netherlands), was used to take the recordings, using the following settings: smoothing/spline: 6, integration time: 150, pluses/integration: 15, average over: 10 readings. Data were analysed individually for each transect. The matrix-algebraic procedure described in Thorpe (2002) was followed to extract spectrophotometric data for analysis. This procedure gives several independent wavelength segments (colours) as unit characters which can be compared across large samples of individuals at a large number of localities. The methods used by Thorpe and Stenson (2003) were followed, where: UV 330-380nm, UV/violet 380-430nm, blue 430-490nm, green 520-590nm, yellow/orange 590-640nm, and red 640-710nm. These colour characters from the anterior and posterior dewlap (12 in all) were subjected to a CVA with each transect locality as the pre-defined groups.

2.3 MOLECULAR MARKERS

2.3.1 MITOCHONDRIAL DNA

Mitochondrial DNA is circular organelle DNA that rarely recombines, is generally maternally inherited (Hutchison et al., 1974; Gyllensten et al., 1991) and evolves more rapidly than much nuclear DNA (Brown et al., 1979). It also has a smaller effective population size. These characteristics mean that coalescence is quicker, with less chance of incomplete lineage sorting, making it a good marker for molecular phylogenies (Moore, 1995). Thorpe and Stenson (2003) used one of the genes in the mitochondria, cytochrome *b*, to reconstruct the phylogeny of *A. roquet*. The sequences from this study were aligned and screened for diagnostic polymorphism that discriminated between two lineages. Polymerase chain reaction-based restricted fragment length polymorphism (PCR-RFLPs) was then used for individual assignment to lineage in Chapter 5.
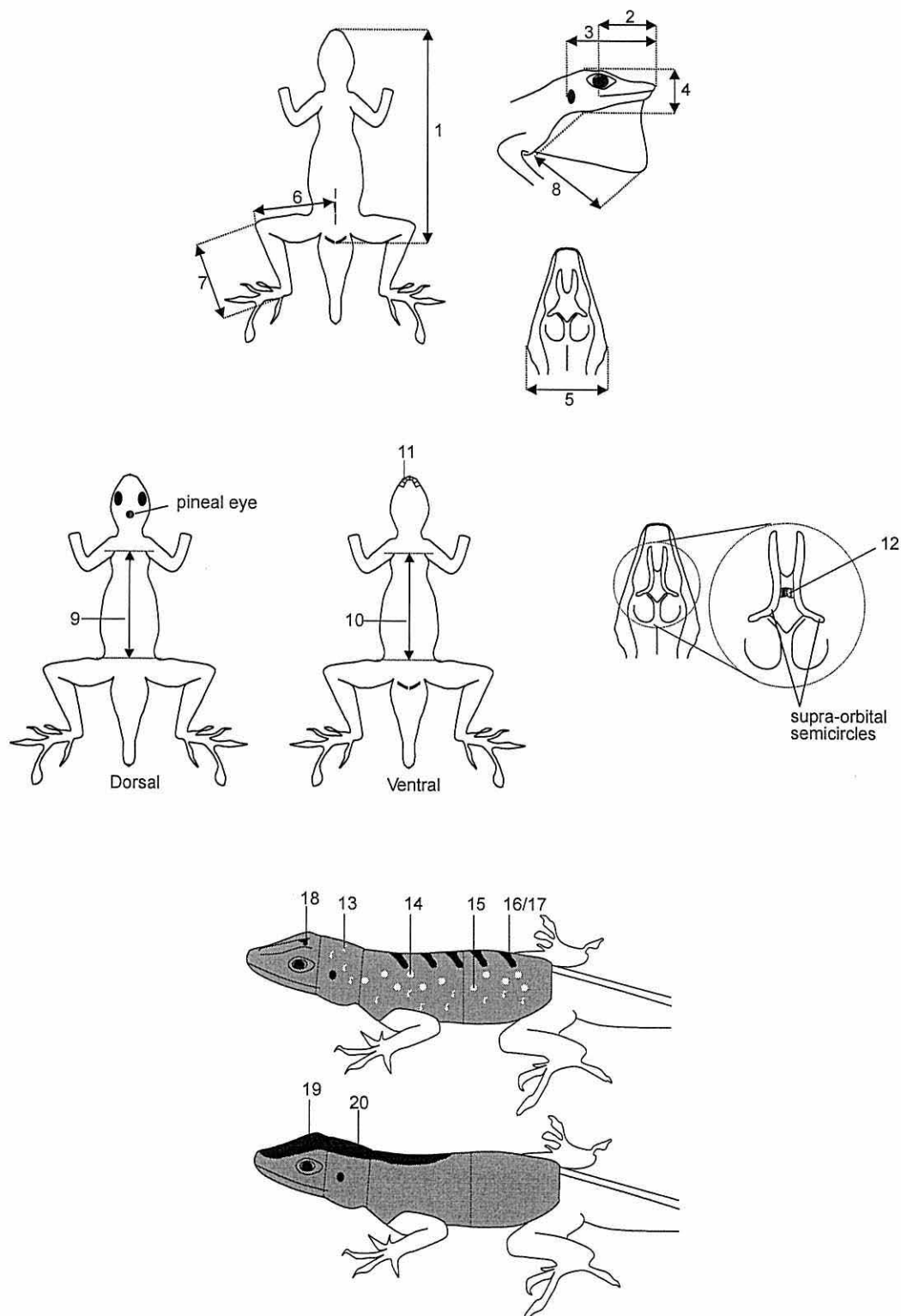
Figure 2.1: Morphological characters for *A. roquet.* Adapted from Ogden (2002). See table 2.1 for details.

2.3.2 MICROSATELLITES

Microsatellites are tandem repeats of short DNA motifs, typically 1-6 bp length (Chambers and MacAvoy, 2000; Toth et al., 2000; Li et al., 2002; Zane et al., 2002). They are also known under the following names: simple sequence repeats (SSR), variable number tandem repeats (VNTR) and short tandem repeats (STR). Microsatellites found in eukaryotes are pure, compound or interrupted (Jarne and Lagoda, 1996) and for population genetic purposes mostly pure di-, tri- and tetra-nucleotide repeats are used (Chambers and MacAvoy, 2000; Selkoe and Toonen, 2006). The most common repeats found in eukaryotes are dinucleotides and the motifs are usually repeated between five and 40 times (Toth et al., 2000).

For this thesis microsatellites were developed for a number of species in the *A. roquet* group of anoles. *Anolis roquet* samples were screened with a combination of markers of which some were developed specifically for *A. roquet* (Ogden et al., 2002; Gow et al., 2006) whereas others were cross-amplified from other species in the group (Gow et al., 2006; Johansson et al., 2008a).

Microsatellite diversity in a species or population is detected by amplifying DNA using the polymerase chain reaction (PCR) with unique primers flanking microsatellites. The resulting DNA fragments are separated according to size using gel electrophoresis. The popularity of microsatellites is due to their relative abundance in eukaryotic genomes, that they can be presumed selectively neutral, that they have a Mendelian pattern of inheritance and that their high mutation rates (10^{-2}-10^{-6} mutations per locus per generation) generate the high level of allelic diversity that is often necessary for population genetic studies (Schlotterer, 2000). Microsatellite data were analysed in Chapter 3, 4 and 6, using nine microsatellites: AAE-P2F9, ABO-P4A9, AEX-P1H11, ALU-MS06, ARO-035, ARO-062, ARO-065, ARO-120, ARO-HJ2.

TESTING THE MARKER: HARDY-WEINBERG EQUILIBRIUM

The initial step in analysing genetic data from microsatellites is to test for departures from the Hardy-Weinberg equilibrium (HWE). Observed genotype frequencies are compared with the frequencies expected for an ideal population. The equation was developed independently by Godfrey H. Hardy and Wilheim Weinberg and published in 1908 (Hardy, 1908). The HWE are based on a number of assumptions:

- a large population size (infinite in theory)

- migration is negligible

- mutation is negligible

- generations do not overlap

- the organism is diploid

- reproduction is sexual

- mating is random

- natural selection does not affect the gene under consideration

In a diploid organism with alleles *A* and *a*, three possible genotypes can occur : *AA, Aa,* and *aa*. If *p* represents the frequency of *A*, and *q* represents the frequency of *a* (*p* + *q* = *1*), genotype frequencies can be written

$$AA : p^2 \qquad Aa : 2pq \qquad aa : q^2$$

From this, the equation for genotype frequencies is given by:

$$p^2 + 2pq + q^2 = 1$$

The equations can be extended for genes with more than two alleles.

A population that deviates from the HWE is likely to be inbreeding or outbreeding, or subject to natural selection. A common deviation from HWE is a heterozygote deficit, which can be an indication of inbreeding, but may also be due to the Wahlund effect. The Wahlund effect may be present if two or more populations are inadvertently sampled as one, either because they co-occur but rarely interbreed, or because the sampling scale chosen is larger than the true scale of a population. As a result there will be more homozygotes than expected under the HWE (Hartl and Clark, 1997).

TESTING THE MARKER: LINKAGE DISEQUILIBRIUM

In multilocus analyses, it is also necessary to test for linkage disequilibrium (LD). Loci are in disequilibrum when a particular allele at one locus is associated with a specific allele at a second locus more often than expected if the loci were segregating independently

in a population (Hartl and Clark, 1997). If there is LD pseudo-replication occurs in any further analyses.

Using the simplest model for two loci, A and B, with two alleles (A, a and B, b) at each locus; P_{AB} represents the observed frequency of the haplotype that consists of alleles A and B. If alleles assort independently at the two loci, the expected halotype frequency is calculated as the product of the allele frequency of each of the two alleles: $P_A \times P_B$, where P_A is the frequency of allele A at the first locus, and P_B is the frequency of allele B at the second locus. A simple measure of linkage, D, is then given by:

$$D = P_{AB} - P_A P_B$$

However, complex statistical procedures are implemented in softwares such as ARLEQUIN, GENEPOP and FSTAT for evaluating disequilibrium from microsatellite markers by searching for correlations between alleles at different loci.

POPULATION STRUCTURE: SUMMARY STATISTICS

Most organisms have significant population substructure, i.e. subpopulations can be grouped into progressively inclusive levels by means of a hierarchy. A hierarchical structure results in a heterozygote deficiency at each level. The inbreeding coefficient, F, estimates this reduction, and is calculated from the values obtained by calculating the HWE:

$$F = 1 - H_O/H_E$$

where H_O = observed heterozygotes, and H_E = expected heterozygotes.

From the inbreeding coefficient F-statistics can be derived. Wright developed the theory of F-statistics to utilise allele frequency data and estimate gene flow and population subdivision based on an infinite island model (Wright, 1931, Wright 1953). To begin hierarchical measures of heterozygosity are defined as: H_I = mean observed heterozygosity per individual within subpopulations, H_S = mean expected heterozygosity within random mating subpopulations ($2p_i q_i$) and H_T = the expected heterozygosity in the randomly mating total population ($2pq$).

F_{IS} or inbreeding coefficient, can then be calculated as:

$$F_{IS} = (H_S - H_I)/H_S$$

F_{ST} or fixation index, can be calculated as:

$$F_{ST} = (H_T - H_S)/H_T$$

F_{IT} or overall fixation index, can be calculated as:

$$F_{IT} = (H_T - H_I)/H_T$$

F_{IS} is the inbreeding coefficient of an **I**ndividual to the **S**ubpopulation, and can range from -1.0 (all individuals are heterozygous) to =1.0 (no observed heterozygotes). F_{IS} is also referred to as F. F_{ST} is the effect of **S**ubpopulations compared to the **T**otal population, and hence measure the extent of genetic differentiation among subpopulations. It ranges between 0.0 (no differentiation) to 1.0 (complete differentiation). It is also referred to as theta (θ). F_{IT} is the inbreeding coefficient of an **I**ndividual relative to the **T**otal population.

The relationship between the F-statistics is:

$$1 - F_{IT} = (1 - F_{IS})(1 - F_{ST})$$

In a population at equilibrium, i.e. undifferentiated, all F-statistics have a value of 0.

F_{ST}, or its estimator θ (Weir and Cockerham, 1984) calculated as:

$$F_{ST} = \sigma_h^2/\sigma_T^2$$

where $\sigma^2{}_a$ is the variance due to differences in mean allele frequency between subpopulations, and $\sigma^2{}_T$ is the total variance, is the statistic most commonly used for calculating population structure using microsatellite data. The basic calculations to obtain the F-statistics have been modified to accommodate multiple alleles and loci, and sampling effect (Weir and Cockerham, 1984). R-statistics were developed to accommodate mi-

crosatellite mutation models (Slatkin, 1995), however, θ is more commonly used, because of the robustness of the underlying infinite alleles model (IAM) (Balloux and Lugon-Moulin, 2002; Selkoe and Toonen, 2006).

As mentioned above, Wright's F_{ST}'s are based on the infinite island model, in which each island represent a population capable of receiving migrants from other islands, and migration is equally restricted between all islands. Each population has N individuals and a proportion, m, of the individuals are immigrants. Under migration-drift equilibrium F_{ST} provides an indirect measure of immigration in the form, $N_e m$, where N_e is the effective population size of the of the population or subpopulations, and m is the number of immigrants. From (Wright, 1943) formula for a diploid locus:

$$F_{ST} \approx 1/4N_e m + 1$$

However, there are several assumptions for this relationship that are rarely met in practice:

1. all populations are stable in size

2. all populations have $N_e m$ individuals arriving as migrants per generation

Hence estimation of $N_e m$ like this should be interpreted with care (Whitlock and McCauley, 1999; Balloux and Lugon-Moulin, 2002), because mutation is not always negligible compared to migration.

MEASURING BIASED DISPERSAL

Organisms may vary in dispersal pattern according to, for example, size, sex or parasite load. Traditional summary statistics can be estimated separately for each group (e.g. small vs. large) and the estimated values can be tested using t-tests to detect significant differences in dispersal pattern (Goudet et al., 2002).

Bias can also be estimated using the original formulation of an assignment index. Paetkau et al. (1995) computed expected genotypic probabilities from samples from each potential source population and assigned genotypes to the population in which that genotype is most likely to occur. For each individual j in locality k the probability P_{kij} that its genotype at locus i occurs in the kth sample is calculated as the squared frequency of the allele if the individual is homozygous, or, if the individual is heterozygous, twice the the frequencies

of the alleles. If there is no linkage disequilibrium, the probability of occurrence of a multilocus genotype is the product of the probabilities of the individual loci. To ensure that the comparisons can be made between populations, it is necessary to control for different levels of genetic diversity in different populations by taking the probability of the sample and subtract the average probability across individuals in the populations. The corrected assignment index of individual j in sample k then becomes:

$$\mathbf{Alc}_{kj} = \log \left[\prod_{i=1}^{l} P_{kij} \right] - \frac{1}{n} \sum_{j=1}^{n} \log \left[\prod_{i=1}^{l} P_{kij} \right]$$

and is centred around 0, where positive values indicate local genotypes. For a population the mean of the assignment index (mAIC) is used. Assumptions of HWE apply to these methods, i.e. no gametic linkage, random sampling and populations need to be pre-defined . Favre et al. (1997) extend this method further, by estimating the variance of the assignment index (vAIC). These methods are applied in Chapter 4.

POPULATION STRUCTURE: BAYESIAN ASSIGNMENT METHODS

In Chapter 6 hypotheses of restricted gene flow or population substructuring are tested, and patterns compared to quantitative traits and dewlap hue variation (see below). Bayesian assignment methods are a relatively new development for the estimation of population substructuring. These methods originate in the calculation of assignment indices (see above) but have been modified so that populations need not be pre-defined. Using a Bayesian approach and a genetic inheritance model to minimise HWE and linkage disequilibrium within clusters, discrete genetic clusters can be revealed from genotype data (Pritchard et al., 2000). STRUCTURE, the software that was used in this thesis, is one of the most widely used softwares for Bayesian assignment. Other fully Bayesian and part-Bayesian assignment softwares have been developed, for example BAPS (Corander et al., 2003) and PARTITION (Dawson and Belkhir, 2001), however STRUCTURE carries the advantage by performing very well even at low levels of differentiation (Latch et al., 2006).

To detect the number of subpopulation using STRUCTURE, a putative number of populations (for example $K = 2$) is first selected by the user, together with an admixture model. The default admixture model allow for mixed ancestry of individuals, and appears to be appropriate for use in most situations (Pritchard et al., 2000; Latch et al., 2006), but can

be modified by the user. Posterior probabilities are sampled using a Markov Chain Monte Carlo (MCMC) method, discarding the first 10000 or so iterations ('burn-in'). The performance of the dataset is assessed by using diagnostic tools: time series plots of F_{ST}, and LnPD. These are monitored to ensure that the log likelihood stabilises after the burn-in and remains stable during the following MCMC iterations. If stability of the log likelihood is not achieved, burn-in and MCMC iterations are increased.

Once the log likelihood is stable, an increasing number of putative populations (K) are tested until all possible populations, plus one, have been estimated. Each K is run multiple times, to ensure consistent estimations. The number of clusters (populations) is inferred by comparing the posterior probabilities for different K, and by applying an ad-hoc test such as that developed by (Evanno et al., 2005) for use with the program STRUCTURE. Additionally, in the output from STRUCTURE, each individual is given a probability of belonging to the estimated populations. Individuals can be sorted by their probability to give a representation of the distribution of 'population genotypes'.

2.4 CLINE FITTING

Cline fitting is a method that has been widely employed to model character clines in hybrid zones (Szymura and Barton, 1986, 1991; Brumfield et al., 2001; Dasmahapatra et al., 2002; Sites et al., 1995; Leache and Cole, 2007; Babik et al., 2003). If linear transects are sampled across secondary contact zones many genetic and phenotypic characters can be fitted to a tanH cline (sigmoid curve), which is given by:

$$y = \frac{1 + tanh[2(x-c)/w]}{2}$$

where x corresponds to a location along a transect and y correspond to the population means of the character, c is the constant for the position of the cline centre, and w is the constant for the cline width (Szymura and Barton, 1986). The program ANALYSE1.3 implements a maximum likelihood approach to estimate width and centre from genotype or character data (Barton and Baird, 1999). Four parameters are needed; P_{max} and P_{min}, maximum and minimum gene frequency values at the tail ends of a cline; w, width, and c, centre of cline. The maximum likelihood for the cline space is explored by searching for centre and width along a best fit axis. Hypotheses of more complex clines, for example stepped clines, may be explored using a large number of sampling points

If a number of clines are estimated for different characters, statistically significant concordance (width) and coincidence (centre) of clines can be tested. The 95% confidence interval is estimated at points with a two unit decrease of likelihood below the maximum (Edwards, 1972). If centre and width values from one cline fall within two likelihood units of the centre and width from another cline, clines concord and coincide.

MICROSATELLITE DEVELOPMENT

3.1 ABSTRACT

Microsatellites are commonly used genetic markers in population genetic studies. Twenty-two microsatellite markers were developed *de novo* from the *roquet*-series of anoles that inhabit the Lesser Antilles. On average the microsatellites showed high levels of genetic diversity (average 18.28 locus/allele for *Anolis roquet, A. bonairensis, A. trinitatis A. richardii, A. extremus* and *A. aeneus* and 16.8 for *A. luciae*). The markers conformed to Hardy-Weinberg expectations, and there was no evidence of linkage disequilibrium. These markers can be applied to population genetic screening in the species from which they were isolated, and some of the markers are also expected to cross-amplify in other anoles from the *roquet*-series.

3.2 INTRODUCTION

Two primer notes:

- Johansson, H, Surget-Groba, Y, Gow JL, and Thorpe, RS. Development of microsatellite markers in the St Lucia anole, *Anolis luciae*. *Molecular Ecology Resources*, **8**, 1408-1410.

- Gow JL, Johansson H, Surget-Groba Y, Thorpe RS (2006). Ten polymorphic tetranucleotide microsatellite markers isolated from the *Anolis roquet* series of Caribbean lizards. *Molecular Ecology Notes*, **6**, 873-876.

were produced and accepted for publication from this work, and these form part of the submission of this thesis (Appendix D).

Microsatellite markers are commonly used to obtain genetic data for empirical studies of divergence and speciation at fine taxonomic levels. Cross-amplification between closely related species may be possible, however the variability of the flanking regions means that primer sites are rarely conserved among distantly related species (Pepin et al., 1995; Primmer et al., 1996; Zhu et al., 2000; FitzSimmons et al., 1995), hence *de novo* isolation is often required for a species of interest.

There are different strategies for microsatellite isolation (Glenn and Schable, 2005). The basic approach involves cloning small genomic fragments and using labeled oligonucleotide probes of microsatellite repeats to identify clones with microsatellites. This works well in organisms in which microsatellites are abundant (Tautz, 1989). For those organisms in which microsatellites are rare, or when less common repeats are required (e.g. tetranucleotides), two main classes of enrichment protocols have been developed; uracil-DNA selection (Ostrander et al., 1992) and hyridization capture (Armour et al., 1994; Kandpal et al., 1994). Hybridization capture is faster and easier to do than uracil-DNA selection, and allows selection of the selected motif before cloning, hence, it is the most commonly used method (Glenn and Schable, 2005).

To complement dinucleotide microsatellites previously developed by Ogden et al. (2002), we isolated microsatellites for the *roquet*-series of anoles. We used a protocol by Gardner et al. (1999) that was developed for isolation of microsatellites for the lizard *Egernia stokesii*. This protocol utilizes the affinity of biotin to form strong bonds with streptavidin to facilitate selective hybridization and microsatellite recovery. We chose to enrich for tetranucleotide repeats as they are widely considered to suffer less slippage error and to be less ambiguous to score than dinucleotide repeats (Schlotterer and Tautz, 1992).

Tetranucleotide microsatellites were isolated two times: the repeat motifs TAGA, AAAG, TCAG and TACA were isolated for *A. roquet, A. bonairensis, A. luciae, A. extremus* and *A. trinitatis*. After the first isolation it was found that the repeat motif TAGA was the most sucessful, hence only this motif was used during the second isolation for *A. richardii* and *A. aeneus*. The results for *A. roquet, A. bonairensis, A. richardii, A. aeneus* and *A. trinitatis* from the first two isolations were published in one primer note (Gow et al., 2006). The results for *A. luciae* were published in a second primer note (Johansson et al., 2008a). At the screening stage two different methods were used: one for *A. luciae* and a different method for the remaining species. We also cross-amplified some of the isolated microsatellites in anoles in the same series, to evaluate their usefulness in closely related species (Gow et al., 2006; Johansson et al., 2008a).

3.3 METHODS AND MATERIALS

Prior to isolation linker oligos S61: 5' - GGC CAG AGA CCC CAA GCT TC - 3', and phosphorylated (at 3' end) S62: 5' - GAT CCG AAG CTT GGG GTC TCT GGC C - 3' were synthesized. The S61 oligo blocks terminal priming sites to limit formations of concatamers. The chosen tetranucleotide repeat motifs (AAAG, TCAG, TACA and TAGA) were synthesized as oligonucleotides with the motif repeated six times and a non-complementary region with biotin at the second base from the 3' end (5' - $(AAAG)_6$GCAC [biotin]A - 3') for use with magnetic capture, and they were also synthesized separately without biotin (5' - (repeat motif)$_6$ - 3'), for use in clone screening. All oligonucleotides were synthesized by MWG Biotech (Germany).

The following solutions were prepared ahead of the isolation:

1x SSC = 0.15M NaCl, 15mM trisodium citrate

1x hybridization solution = 0.5 M NaCl, 4 % w/v polythelene glycol 8000)

0.15 M NaOH

10xTE, pH 8.0

Tris-HCl, pH 8.0

3.3.1 DNA EXTRACTION, RESTRICTION, LIGATION TO THE ADAPTER AND FRAGMENT SIZE SELECTION

DNA was extracted from eight tailtips from each species using Qiagen DNEasy Blood &Tissue (Qiagen) kit following instructions for mouse tail. For each species 5 ng of DNA was pooled from equal amounts of the extracted DNA. The DNA was digested in a 20 µl reaction containing 5 units of the restriction enzyme *Sau*3A at 37 °C for 3 hrs followed by incubation at 95°C for 30 min to inactivate the enzyme.

An adapter was generated by hybridizing 1.5 nmol S62 with equal amounts of S61 in a heating block set to 80°C, and subsequently allowed to cool slowly for one hour at room temperature. Five µl of the *Sau*3A-restricted DNA was ligated to 0.9 nmol of the adapter in 1x DNA ligase buffer with 40 units of T4 DNA ligase (Promega) in a final volume of 200 µl and incubated for 5 min at room temperature. The reaction mix was slowly cooled in a 4°C waterbath overnight. The following morning DNA was ethanol precipitated and resuspended in 20 µl TE. The suspension was left for at least an hour before being run out alongside a 100 bp ladder on a 2.5% microsieve agarose gel stained with ethidium bromide. Bands between 200 and 1000 bp were excised from the gel under UV illumination, and DNA recovered from the gel using the Qiagen Gel Extraction kit (Qiagen) and eluted in 50 µl of water.

3.3.2 MAGNETIC ISOLATION OF TETRANUCLEOTIDE REPEATS

The eluted DNA was then subject to magnetic capture using the biotinylated microsatellite motifs and Streptavidin MagneSphere®Paramagnetic Particles (SA-PMPs) (Promega). One-hundred microlitres of SA-PMPs per biotinylated motif were washed and suspended following the manufacturers instructions and then re-suspended in 100 µl 5 x SSC containing 200 pmol of the biotinylated microsatellite motifs.

The bead mixture was incubated for 15 min at room temperature and then washed three times in 5 x SSC and re-suspended in 50 µl of 1x hybridization solution held at 50°C.

Twenty pmol of S61 and 40 µl of 1x hybridization solution were mixed in a new tube and 10 µl of the ligated DNA/adapter solution was added. This solution was heat denatured for 5 min at 95°C followed by cooling to 55°C before adding all of the resuspended bead mixture and incubating at 55 °for 20 min. The beads were then washed eight times in 100 µl of 1 x SSC with 10 pmol of S61 linker oligo to remove unbound DNA fragments as

follows:

- four times at 40°C

- four times at 50°C

The captured DNA fragments were then eluted from the beads by denaturation at room temperature for 20 minutes in 20 μl 0.15 M NaOH, and neutralized with 1.3 μl 1.25 M acetic acid and 2.2 μl 10 x TE (pH 8.0). The DNA was then purified using the QIAquick PCR purification kit, and eluted in 50 μl Tris-HCl (pH 8.0).

3.3.3 AMPLIFICATION OF CAPTURED DNA, CLONING AND COLONY SCREENING

Captured fragments were amplified in 50 μl reactions using 10 x buffer, 0.5 units of *Taq* polymerase, 2.5 mM each dNTP, 4 mM MgCl$_2$ (Promega) and 60 pmol S61. PCR reactions were performed with a amplification profile of 94°C for 4 minutes, followed by 60°C for 45 s, 72°C for 1 min and then 34 cyces of 94°C for 45 s, 60°C for 45 s and 72°C for 1 min, and a finally 72°C for 5 min. Amplified reactions were kept at 4°C until visualised on a 1% agarose gel.

After visualisation the amplified product was cloned into *Escherichia coli* using TOPO TA cloning kit (Invitrogen) with PCR®2.1.TOPO® plasmid vector and One Shot ®TOP 10F *E. coli* chemically competent cells using the manufacturers instructions. The clone mixture was plated out on autoclaved Luria Bertani (LB) plates made with 10 g/l tryptone, 5 g/l yeast extract, 5 g/l NaCl and 15 g/l agar, with ampicillin added to concentration of 50 g/l. Plates were left to set and then coated with X.gal (40 mg/ml) and IPTG (100 mM) and left to dry at 37°C, at which temperature they were kept until used. Thirty-five μl of clone mixture was distributed evenly across each LB plate using purpose-made sterile glass spreaders, in a laminar flow chamber.

The vector contains the sequencing primers M13-20F and M13R binding sites for PCR amplification. Cells in which the vector has been inserted are resistant to the antibiotic in the growth medium. In addition, successfully inserted DNA disrupts the *lacZ* gene that produce beta-galactosidase. The IPTG is an inducer of beta-galactosidase in cells and cleavage of X.gal by beta-galactosidase leads to the oxidation of an insoluble blue product, hence cell colonies that do not have foreign DNA inserted turn blue.

Plates were incubated over night at 37°C, and then kept at 4°C. White colonies were

picked with sterile sticks and immersed in 20 µl Tris-HCL (pH 8.5) on 96-well PCR plates and incubated for 10 minutes at 95°C.

3.3.4   PCR SCREENING OF COLONIES AND PRIMER DESIGN

Colonies were screened using 10 µl PCR reactions (10 x Promega Buffer, 0.1 U *Taq*, 4mM MgCl$_2$, 200 µM dNTPs, 0.2 µM each of primers M13-20F and M13-R, and 0.2 µM of motif primer) using the same PCR program that was used to amplify the captured fragments. PCR products were visualised on 1.5% agarose gel viewed on a UV transillumination. Inclusion of vector primers (M13-20F and M13-R) and a third non-biotinylated primer with the motif used for selection into the same mix promotes two parallel reactions. If a clone contains a repetitive element the cloned inset corresponding to the region amplified by the M13 primers produce one band, and the actual motif itself produces either another band or a smear when the PCR products are visualized on agarose gel under UV illumination.

In the first two isolations PCR products with double bands were purified using Qiagen PCR purification kit and then sequenced (using only the M13 primers) by Macrogen (www.macrogen.com), in the final isolation all amplified clones were sent for sequencing by Macrogen.

Microsatellite sequences were viewed with BIOEDIT (http://www.mbio.ncsu.edu/BioEdit/ BioEdit.html) or CODONCODE (CodonCode Corporation: www.codoncode.com) sequence aligners, and for those sequences that contained a repeat motif and had adequate flanking regions primers were designed using the web-based program PRIMER3 http://frodo.wi.mit .edu/). PRIMER3 generates a number of possible primers for each submitted sequence. We used the default parameters of the program (primer length:18- 27 bp with an optimum of 20 bp, primer melting temperature (T_M): 57-63 °C with an optimum of 60 °C, and a GC content between 20% and 80%) with the exception of an added G-C clamp (stronger bonds at the end of the primer) and we preferentially chose primers that were close to the target sequence.

The forward primers for *A. luciae* also incorporated an M13 tail, which allows for the use of FAM labelled universal M13 primers for amplification (Oetting et al., 1995), which can reduce costs of screening. A pigtail (GTTT) was also added at the 5' end of the reverse primer, to reduce stutter and improve reliability of allele scoring (Brownstein et al., 1996).

All primers were ordered from and synthesized by MWG-Biotech (http://www.mwg-biotech.com/).

3.3.5 Amplification and genotyping of microsatellites isolated from *A. roquet, A. bonairensis, A. trinitatis A. richardii, A. extremus* and *A. aeneus* and statistical analysis of genotype data

The primers were first tested on six individuals, from the species from which they were derived. Loci were amplified in 10 µl reactions using 5 ng of template DNA, 1.5-3 mM $MgCl_2$, 0.2 mM of each dNTP, 0.05 µM of each primer (forward labelled with CY5 or CY5.5 dye) 0.5 U of *Taq* DNA polymerase and 10 x buffer (Pomega). The reactions were subject to denaturation at 94°C for 2 min, followed by 30 thermal cycles of 30 s at 94°C, 30 s at a locus-specific annealing temperature (Table 3.1), and 30 s at 72°C, and a final extension of 5 min at 72°C. Amplified locus were then run out on a CEQ 8000 Genetic Analyser (Beckmann Coulter) together with a internal size standard, the CEQ DNA Size Standard Kit-400.

Those loci that yielded reproducible and easily interpretable bands were first screened on eight *A. roquet* individuals from three mtDNA lineages (Thorpe and Stenson, 2003), and those loci that were found to amplify consistently were then screened on 96 individuals from two disparate populations. Reliable loci were also cross-amplified in seven species from the *roquet*-series of anoles and bands were visualised on agarose gel (Table 3.2).

Observed and expected heterozygosities were calculated with the Microsoft Excel add-in MICROSATELLITE TOOLKIT (Park (2001): http://www.animalgenomics.ucd.ie/sdepark/ms-toolkit/), linkage disequilibrium (LD) and and Hardy-Weinberg equilibrium (HWE) were tested in GENEPOP v 3.3 (Raymound and Rousset (1995): http://genepop.curtin.edu.au/) and a Bonferroni correction applied to correct for multiple comparisons. An estimate of population differentiation between the two sampled population, F_{ST}, was also calculated using GENEPOP v.3.3.

3.3.6 AMPLIFICATION AND GENOTYPING OF MICROSATELLITES ISOLATED FROM *A. luciae* AND STATISTICAL ANALYSIS OF GENOTYPE DATA

Four *A. luciae* individuals were initially used to test the microsatellite primers. Loci were amplified using 5 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.05 µM of the forward primer, 0.5 mM of the reverse primer and of the labelled primer, and 0.5 U of *Taq* DNA polymerase with 5 x buffer (Promega). The profile of amplification was 2 min at 95°C, followed by 29 cycles (30 s at 95°C, 30 s at 55°C and 30 s at 72°C) and a final extension period of 5 min at 72°C. Samples were then run on an ABI3130*xl* Genetic Analyzer with the internal size standard LIZ-600. Those loci that amplified reliably were then screened on 32 individuals from one *A. luciae* population. All primers from the previous two isolations (Table 3.1) were also amplified on *A. luciae* using the conditions described in Table 3.1, and genotyped as described above.

Observed and expected heterozygosities were calculated using MICROSATELLITE TOOLKIT (Park, 2001), linkage disequilibrium (LD) and Hardy-Weinberg equilibrium (HWE) were tested in ARLEQUIN v3.01(Schneider et al. (2000): http://lgb.unige.ch/arlequin/) and a sequential Bonferroni correction applied to correct for multiple comparisons (Rice, 1989).

3.4 RESULTS

3.4.1 RESULTS FOR *A. roquet, A. bonairensis, A. trinitatis A. richardii, A. extremus* AND *A. aeneus*

A total of 144 clones were isolated and were sent to Macrogen for sequencing. Of these clones a total of 93 contained microsatellite repeats. However after excluding duplicates and those sequences that had insufficient flanking region for primer design, a total of 55 unique microsatellites remained (38% of clones), for which primers were designed. Ten microsatellites were found to amplify reliably, were polymorphic and did not show obvious evidence of null alleles. Five of these microsatellites were pure tetranucleotide repeats, two (AexP2E3 and AtrP16.55) were compound microsatellites and the remaining three were interrupted repeats (AexP4H6 and AriP2D8) (Table 3.1). The shortest allele amplified was 97 basepairs (AtrP16.55) and the longest allele 371 basepairs

(AboP4A9).

Genetic diversity was found to be high in the 10 microsatellites. The number of alleles per locus ranged from nine to 22, averaging 18.28 over all loci in the screening of 96 *A. roquet* individuals. Gene diversity and observed heterozygosity per locus (averaged over the two samples) ranged from 0.51-0.91 (mean = 0.81) and from 0.46-0.89 (mean = 0.74), respectively (Table 3.1).

No evidence of significant linkage disequilibra was found after Bonferroni correction. With the exception of one locus, AexP4H6, which was found to have a heterozygote deficit in one population, all loci conformed to HWE expectations following Bonferroni correction. F_{ST} estimates ranged from 0.01-0.45 per locus, with a significant global F_{ST} of 0.10.

Many of the loci also appeared to successfully amplify in related species (Table 3.2).

Table 3.1: Polymorphic microsatellite loci for the *Anolis roquet* series. Locus name prefix indicates the species of origin: Aae = *A. aeneus*; Abo = *A. bonairensis*; Aex = *A. extremus*; Ari = *A. richardii*; Aro = *A. roquet*; Atr = *A. trinitatis*. The repeat motif and GenBank Accession number of the sequenced clones are given. Primer sequences, annealing temperature (T_a in °(C) and MgCl$_2$ concentration (MgCl$_2$ (mM)) for optimal PCR amplification in the species of origin are given for each locus, alongside the number (N) and range (in base pairs) of alleles found among six specimens of the species of origin sampled from different localities. Number of alleles, allele size range, and expected (H_E) and observed (H_O) heterozygosities per locus averaged over two sampled populations are described for the eight microsatellites that were polymorphic within *A. roquet* (n = 96 from two disparate populations from Martinique).

Locus	Repeat motif	Primer sequence (5'-3') (F, forward; R, reverse)	T_a (°C)	MgCl$_2$ (mM)	N	Allele size range (bp)	N	Allele size range (bp)	H_E	H_O	GenBank Accession no
							A. roquet screening (N=96)				
AaeP2F9	(CTAT)13	F: CAATGTTTTGCTCTTGCTATTT R: GGCTGATTTGTCCTTTCTGG	55	2.5	5	219-243	16	223-281	0.86	0.89	DQ379371
AaeP2F5	(CTAT)8	F: GCAAAGGCAATAGGAAAAGG R: GTTGGCGATGTCCCATAAAC	55	1.5	9	268-326	15	272-352	0.86	0.84	DQ379372
AboP4A9	(CTAT)9	F: GTGACTATGAAGGGGAATCTTG R: GATGTAGGCTTTGCTGCTGT	55	1.5	4	359-371	12	335-365	0.51	0.46	DQ379373
AexP2E3	(CTAT)13(AC) 8	F: TCTTCCTCCCTTTCCCAGAT R: TAGCTTCCCCTTTTGCTTTG	55	2.5	8	207-257	18	211-263	0.86	0.78	DQ379374
AexP1H11	(CTAT)11	F: GCTATCCATCCATCATTTCTATGT R: AAACTGTAATTCCCAAGATTCCA	50	3.5	7	273-303	20	249-301	0.91	0.83	DQ379375
AexP4H6	(CT/CAT)17	F: TCTGGGTTTTCTGGAAGCTG R: TCAAACCATGTAGGAACCTGTG	53	3.5	7	167-217	22	171-231	0.90	0.74	DQ379376
AriP2D8	(CT/CAT)24	F: GGAGCAGAAAGAGAAGAAACATC R: TCAAACGGGAAAACAAGAAC	53	3.5	3	227-307	NA	NA	NA	NA	DQ379377
AroHJ2	(TAGA)10	F: ACATGAATGGTGGGAG R: TTGACCACACTCTGATGTTGC	60	1.5	4	218-226	9	210-242	0.77	0.70	DQ379378
AroHJ5	(TAGA)11	F: TCTTGGAGAAAAGGCAGAAAG R: CTGGAGGCCTACACTATGTCC	55	3	4	211-223	16	187-273	0.84	0.70	DQ379379
AtrP16.55	(CTAT)6 CAT (CTAT)12 CGT(CTAT)6	F: GATAGTGGGCTGGGGAGAG R: CCCGCTCCTGAGATAGATTG	50	3.5	11	97-149	NA	NA	NA	NA	DQ379380

Table 3.2: Cross-species amplification of 10 microsatellite primer pairs within the *Anolis roquet* series. Two samples per species were screened for PCR amplification of a well-defined band in the expected size range (+, presence, - , absence), using conditions listed in Table 3.1 and visualised on 2 % agarose.

Locus	Anole						
	A. aeneus	*A. bonairensis*	*A. extremus*	*A. griseus*	*A. richardii*	*A. roquet*	*A. trinitatis*
AaeP2F9	+	−	+	+	+	+	+
AaeP2F5	+	−	+	+	+	+	+
AaeP2E3	−	−	+	+	−	+	+
AboP4A9	−	+	−	+	+	+	−
AexP1H11	+	+	+	+	−	+	−
AexP4H6	+	+	+	+	+	+	−
AriP2D8	+	+	+	+	+	+	+
AroHJ2	−	+	+	−	+	+	−
AroHJ5	+	+	+	+	+	+	+
AtrP16.55	+	+	+	+	+	−	+

3.4.2 RESULTS FOR *A. luciae*

From the isolation for *A. luciae* a total of 134 clones were sent to and sequenced by Macrogen, and from these 27 had unique microsatellite motifs and adequate flanking region for primer design (20% of clones). Thirteen loci amplified reliably and showed polymorphism in the four samples that initially were screened. One microsatellite was a dinucleotide repeat (ALU-P8C7) and the remaining microsatellites were pure tetranucleotide, predominantely AAAG, repeats (Table 3.3).

The shortest allele amplified was 91 basepairs (ALU-PE12), and the longest allele was 351 basepairs (ALU-MS10) and there was extreme length variation (a minimum of approximately 100 bp difference between the shortest and longest allele) in two loci (ALU-MS02 (118bp) and ALU-P8C7 (99bp)), however these loci did not deviate from HWE and the outlying alleles were found in several individuals.

Of the ten microsatellites developed for *A. roquet, A. bonairensis, A. trinitatis A. richardii, A. extremus* and *A. aeneus* (Table 3.1), one (ABO-P4A9) was found to amplify reliably in *A. luciae* and was also in HWE.

In total, 13 loci (including ABO-P4A9) were found to reliably amplify in *A. luciae*. Genetic diversity was high, number of alleles per locus ranged from seven to 28 and averaged 16.8 across all loci. Mean H_E was 0.87 with locus specific values varying from 0.58 to 0.96 (Table 3.3). There were no significant departures from linkage disequilibrium or HWE following sequential Bonferroni correction.

Table 3.3: Polymorphic microsatellite loci for *Anolis luciae*. Locus name prefix indicates the species of origin: Abo = *A. bonairensis*; Alu = *A. luciae*. Final PCR conditions for all primers were: 1.5mM MgCl$_2$, annealing temperature: 55°C. Primer name, repeat motif and GenBank Accession number of the sequenced clone are given alongside allele size range, number of alleles (N), observed (H$_O$) and expected (H$_E$) heterozygosities for the 13 microsatellites that were polymorphic within *A. luciae* (n = 32 from a single population from St. Lucia).

Locus	Repeat motif	Primer sequence (5'-3') (F, forward; R, reverse)	Allele size range (bp)	N	H$_O$	H$_E$	GenBank Accession no
ALU-MS02	(AAAG)$_{16}$	F: GAAATGCAGCTTCGATCACA R: GTTTATTGGGAGAAGTGGGTTGC	177-316	21	0.78	0.94	EU379658
ALU-MS04	(AAAG)$_{15}$	F: TCAGTCTAAGGGTGGGAGGA R: GTTTGCTCATTAGGATTTGGGACTT	272-327	14	0.84	0.89	EU379659
ALU-MS06	(TAGA)$_{10}$	F: CCTGATGCGCACAAAGAATA R: GTTTTCAAGTCTGGCAATGGA	240-284	12	0.87	0.86	EU379660
ALU-MS10	(AAAG)$_8$	F: GGCTCTTGGCACCTGATAAA R: GTTTCCAATCCTGGCAAAACATCT	252-351	10	0.78	0.82	EU379661
ALU-MS12	(TACA)$_5$	F: TACATACACCGTTGCCCACA R: GTTTATCAGCACACACCAGTCAGC	127-151	7	0.56	0.58	EU379662
ALU-P8A3	(AAAG)$_{13}$	F: GCTGGAAAGATTAACAAAGATGG R: GTTTCCCAACAAAAAGGATTCTGAC	213-268	17	0.78	0.89	EU379663
ALU-P8B10	(AAAG)$_{10}$	F; CAGAGAGTTCAAAAGGAATTGTCC R: GTTTACTGCCTTTCCCTTATGGTC	135-176	28	0.88	0.95	EU379664
ALU-P8C7	(GT)$_7$	F: TCAATGAATGGGCTGGTGT R: GTTTGGAAAGTGTTTCGCTTGA	194-312	14	0.81	0.83	EU379665
ALU-P8C9	(AAAG)$_{17}$	F: TCACTAAATGCCTCTAAGCTATTG R: GTTTCTCCCAAAGGCAAGGTTTC	217-274	16	0.81	0.91	EU379666
ALU-P8E12	(AAAG)$_{15}$	F: TCCTGGACCCATGTGAAAAG R: GTTTAAACAGGAGGCGAAGTTGG	91-125	21	0.94	0.92	EU379667
ALU-P8H7	(AAAG)$_{11}$	F: GGGGGTTCTGTGAATTGTTG R: GTTTCCAAGGTATTCTTCCATTTGC	98-152	17	0.94	0.92	EU379668
ALU-P8H8	(AAAG)$_{12}$	F: GGCATCTCCATTTTAACAAGAAAG R: GTTTGACAGATTTTCCTAGTTCCTCCTG	111-190	26	0.94	0.96	EU379669

3.5 DISCUSSION

These two microsatellite isolations yielded a total of 22 new microsatellites from seven species in the *roquet*-series of anoles. The enrichment efficiency (the percentage of clones that contain microsatellites) was 20% in the *A. luciae* enrichment and 38% in the isolations of the other anoles). This was higher than the rate of 16.7 % that Gardner et al. (1999) reported for *Egernia stokesii*, and similar to the 20.3% rate reported in *Gallotia atlantica* for which the same protocol was used (Bloor et al., 2006). The discrepancy in enrichment efficiency between our isolations may be due to the change in screening protocol; *A. luciae* clones were sent for sequencing without being screened for double bands on agarose gels.

The number of usable microsatellites (polymorphic and in HWE) obtained here are comparable to other microsatellite isolations obtained with similar protocols in other lizards, and to independent isolations in other *Anolis* lizards: Gardner et al. (1999) recovered seven loci for use in *Egernia stokesii*, Bloor et al. (2006) isolated ten microsatellites for use in *Gallotia atlantica* and Bardeleben et al. (2004) isolated 11 tetranucleotide markers for *Anolis sagrei* from two hybridizations following similar protocols. Microsatellite abundance is species-specific (Chambers and MacAvoy, 2000; Li et al., 2002), hence variation in the number of micrsoatellites isolated is expected.

TAGA (CTAT) was found to be the most successful motif in our first hybridization, however, overall the repeat motif AAAG yielded the highest number of usable microsatellites. The AAAG repeat is cited as more common in eukaryotic genomes than the other microsatellite repeat motifs we chose to enrich for (Toth et al., 2000). AAAG repeats were also enriched for by Gardner et al. (1999); Bardeleben et al. (2004) and Bloor et al. (2006), however, there is considerable unpredictability in which microsatellite repeats will be obtained with any given motif. Pure, compound and interrupted tetranucleotide repeats were recovered from *A. roquet, A. bonairensis, A. trinitatis, A. richardii, A. extremus* and *A. aeneus*, whereas pure di- and tetranucleotide repeats were recovered from *A. luciae*. Similar patterns of pure, compound and interrupted tetranucleotides were obtained by Gardner et al. (1999); Bardeleben et al. (2004) and Bloor et al. (2006) in their respective species.

The microsatellites obtained in this chapter show levels of genetic diversity that appear to be higher than those of eight previously isolated dinucleotide microsatellites in *A. roquet* (individuals screened (N) = 600, number of alleles per locus (A) = 5-19, number of alleles averaged over all loci (M_A) = 11.13 (Ogden et al., 2002). These results are also

comparable or higher than 11 tetranucleotide markers from *A. sagrei* (N = 13, A = 9-14 and M_A = 9.81 (Bardeleben et al., 2004)), six dinucleotide markers in *A.oculatus* (N = n/a, A = 13-25, M_A = 18.8 (Stenson et al., 2000), and six dinucleotide microsatellites in *A. cristatellus* (N = 102 - 136, A = 14-19, M_A = 15.5 (Glor et al., 2007)). Bioinformatic studies of fully sequenced genomes suggests that microsatellite variability ultimately depends on number of repeats, length of repeat and purity of the repeats, with short, pure microsatellites with few repeat lengths being the least variable (Legendre et al., 2007). This may explain why the microsatellites isolated here appear more variable than those of Ogden et al. (2002).

Allele sizes were similar in the three isolations, however two loci from *A. luciae* showed extreme length variation. Both loci conform to HWE and outlying alleles were found in several different individuals, suggesting that they are real alleles. The sample size of 32 individuals is likely to be too small to represent all alleles in these very variable microsatellites, and we expect that genotyping more individuals will uncover new alleles across the size range.

Cross-amplification of microsatellite loci from *A. roquet, A. bonairensis, A. trinitatis, A. richardii, A. extremus* and *A. aeneus* on other members in the *A. roquet* were mainly successful when DNA bands were visualized on agarose gel. However, these loci also need to be screened on sufficient individuals and using a genetic analyser before their utility can be confidently reported. Screening of *A. roquet, A. bonairensis, A. trinitatis A. richardii, A. extremus* and *A. aeneus* primers on *A. luciae* samples using a genetic analyser revealed that several primers amplified reliably, however only one locus was also polymorphic, without evidence of null alleles, and did not violate HWE. This is considerably fewer than expected from the results of the agarose screening on the other anole species.

From the three isolations we recovered 22 microsatellites for seven species that can be used together with previously isolated microsatellites for population genetic screening of anoles from the *roquet*-group. Our enrichment efficiency, number of usable microsatellites per isolation and types of microsatellites obtained are comparable to those obtained for other species of lizards using similar protocols. The microsatellites obtained show high genetic diversity, and we expect that a small proportion of them will also cross-amplify in closely related species within the *roquet*-series of anoles.

MICROSATELLITE DATA SHOW EVIDENCE FOR MALE-BIASED DISPERSAL IN THE CARIBBEAN LIZARD *Anolis roquet*

4.1 ABSTRACT

Dispersal is a key component of an organisms life history and differences in dispersal between sexes appear to be widespread in vertebrates. However, most predictions of sex-biased dispersal have been based on observations of social structure in birds and mammals and more data are needed on other taxa to test whether these predictions apply in other organisms. Caribbean anole lizards are important model organisms in various biological disciplines, including evolutionary biology. However, very little is known about their dispersal strategies despite the importance of dispersal for population structure and dynamics. Here we use nine microsatellite markers to assess signatures of sex-biased dispersal on two spatial sampling scales in *Anolis roquet*, an anole endemic to the island of Martinique. Significantly higher gene diversity (H_S) and lower mean assignment value (mAIC) was found in males on the larger spatial sampling scale. Significant heterozygote deficit (F_{IS}), lower population differentiation (F_{ST}), mAIC and variance of assignment index (vAIC) was found in males on the smaller spatial scale. The observation of male biased dispersal conforms with expectations based on the polygynous mating system of *Anolis roquet*, and contributes to an explanation of the contrasting patterns of genetic structure between maternal and biparental markers that have been reported previously in this, and other anoline, species.

4.2 INTRODUCTION

Dispersal is a key component of an organisms life history, affecting both the evolution and persistence of a species (Clobert et al., 2001). Dispersal influences the rate of differentiation between sub-populations and the degree to which populations function as independent demographic units (Palo et al., 2004). Hence, understanding the dispersal pattern of an organism is a fundamental requirement for accurate inferences about population structure and dynamics. In sexual species, dispersal often occurs predominantly in one of the sexes (sex-biased dispersal). There are three hypotheses that are commonly invoked to explain the disparity in dispersal between the sexes: competition among related females for resources (local resource competition) (Greenwood, 1980), competition between related males for mates (local mate competition) (Dobson, 1982; Perrin and Mazalov, 2000), and avoidance of inbreeding (Pusey, 1987). These hypotheses are not mutually exclusive and share one common facet in that they predict male-biased dispersal in taxa with polygynous mating systems. Conversely, for monogamous species, only local resource competition predicts a bias in dispersal, and this bias is in favour of dispersal among females (Greenwood, 1980). These predictions are broadly supported by empirical evidence from mammals and birds; in mammals (often polygynous), males normally disperse further from their natal area, whereas in birds (often monogamous), female-biased dispersal predominates (Greenwood, 1980; Handley and Perrin, 2007). Nevertheless, there are examples of species of mammals and birds that do not conform to the general patterns (Clarke et al., 1997; Gibbs et al., 2000; Dallimer et al., 2002; Moller and Beheregaray, 2004; Williams and Raebold, 2005; Broquet et al., 2006; Handley and Perrin, 2007), suggesting that mating system hypotheses cannot be applied universally. Moreover, recent studies have suggested that kin selection and sociality may play an important part in the evolution of sex-biased dispersal (Devillard et al., 2004).

Studies on species in other taxa, for example salmonids (Hutchings and Gerber, 2002; Bekkevold et al., 2004; Fraser et al., 2004; Palstra et al., 2007), cichlids (Knight et al., 1999; Taylor et al., 2003), and frogs (Lampert et al., 2003; Austin et al., 2003; Palo et al., 2004) are relatively few and the patterns of sex-biased dispersal are equivocal. Likewise, there are relatively few published studies that examine patterns of sex-biased dispersal in lizards. Monogamy in lizards is relatively rare; however, in the few known examples microsatellite data have revealed that slight female bias in dispersal appears to be the norm (Bull, 2000; Gardner et al., 2001; Stow et al., 2001; Chapple and Keogh, 2005), in line with predictions from mating system and local resource competition. In lizards with polygynous mating systems, patterns of sex-biased dispersal vary. In the common lizard

(*Lacerta vivipara*) a mark-recapture study shows slight male-biased dispersal, where dispersal rates are dependent on female density and kinship (Lena et al., 1998), while microsatellite data showed an indication of male-biased dispersal in *Anolis oculatus* (Stenson et al., 2002) and, from mark-recapture data, in *Sceloporus occidentalis* (Massot et al., 2003). Mark-recapture studies in *Uta stansburiana* showed that males disperse further than females in some years, but not in others (Doughty and Sinervo, 1994a,b). However, mark-recapture data from *Lacerta agilis* suggest that juvenile females disperse further than males (Olsson et al., 1996). Both natal and breeding dispersal are also higher in females of the alpine lizard, *Niveoscincus microlepitodus*, according to mark-recapture data (Olsson and Shine, 2003). Hence, with only a limited number of studies in lizards, no strong general pattern of sex-biased dispersal seems to emerge. Further investigation of sex-biased dispersal in different taxonomic groups is necessary in order to make cross-taxa comparisons with the patterns observed in birds and mammals, and to develop a more general framework for the evolution of sex-biased dispersal.

Anoles have long been recognised as important model organisms in various biological disciplines (Roughgarden, 1995; Schluter, 2000; Lovern et al., 2004; Losos, 2004; Thorpe et al., 2004), but there have been only limited field (Andrews and Rand, 1983) and genetic (Stenson et al., 2002) studies of dispersal in this genus. Here we investigate genetic evidence for sex-biased dispersal in *Anolis roquet*, one of the nine species in the roquet series of anole lizards that inhabit the Southern Lesser Antilles. *Anolis roquet* is an arboreal insectivorous lizard that is endemic to Martinique, and which is found in high densities across most of the island. A recent phylogenetic study on *A. roquet* revealed four very distinct mtDNA lineages that were found to be closely associated with geographical regions (Thorpe and Stenson, 2003). These regions correspond to peripheral precursor islands that were joined by the uplifting of a central region (Andreieff et al., 1976; Bouysse et al., 1983; Maury et al., 1990; Sigurdsson and Carey, 1991). The phylogeographic pattern suggests that young precursor islands were colonised by anoles, and individual *A. roquet* lineages evolved in geographical isolation (allopatry) until the joining of the precursor islands (Thorpe and Stenson, 2003). Following secondary contact, a distinct geographical pattern of mtDNA lineage distribution persists and lineage transitions occur over very short geographical distances (Ogden and Thorpe, 2002; Thorpe et al., 2008). In contrast, nuclear microsatellite data has revealed a pattern of high nuclear gene flow across these secondary contact zones (Ogden and Thorpe, 2002; Thorpe et al., 2008). This type of pattern is commonly seen in organisms with male-biased dispersal (Taberlet and Bouvet, 1994; Gibbs et al., 2000; Waits et al., 2000; Castella et al., 2001; Petit et al., 2001), a hypothesis that has not been tested in *A. roquet*. Given that anoles

generally have polygynous mating systems (Jenssen et al., 2001) predicting male-biased dispersal, it is important to test hypotheses of sex-biased dispersal as a contributing factor to the contrasting patterns between mtDNA and nuclear DNA in this and other anoline species (Stenson et al., 2002).

Direct estimations of dispersal through mark-recapture studies are time-consuming and expensive due to the extensive fieldwork required (Berry et al., 2004). Furthermore, *Anolis roquet* is a small, arboreal, often cryptic animal that inhabits a complex tropical habitat. Hence, it would be particularly difficult to survey dispersing juveniles efficiently. Moreover, in anoles there is a high turnover, particularly among juveniles (Andrews and Rand, 1983), which would require a very high number of individuals to be marked in order to reliably estimate dispersal. An indirect approach based on microsatellite frequencies offers an attractive alternative to mark-recapture studies (Goudet et al., 2002; Prugnolle and de Meeus, 2002; Berry et al., 2004; Handley and Perrin, 2007). Sex-biased dispersal affects genetic structure between and within populations, which can be detected by calculating indices from polymorphic genetic data. Results from these methods based on genetic data have been favourably compared with mark-recapture studies (Favre et al., 1997).

4.3 MATERIALS AND METHODS

Anolis roquet is a sexually dimorphic lizard; mature males are larger than females and show distinctive markings. Both males and females have dewlaps, however the dewlap on males is larger and more brightly coloured (Lazell, 1972), hence discrimination between adults from each sex is straightforward. Only adult lizards were used for this study.

We sampled a total of 17 localities on two spatial scales. Ten island-wide localities (distance between localities ranged between 4-43.6 km, the mean being 17.12 km) were sampled between March and May 2005-2007. Localities were chosen to incorporate at least one locality from each mtDNA lineage and cover as many habitat types as possible. Seven further localities situated along a 4 km transect, all in the same habitat type (Figure 4.1), were sampled between April and May 2007. Distances between these latter localities are likely to be a more realistic scale for juvenile dispersal. Each locality (at both sampling scales) was sampled over no more than two days in the same year. Only data from locations yielding at least 28 adult individuals and approximately equal numbers of females and males were included in this study.
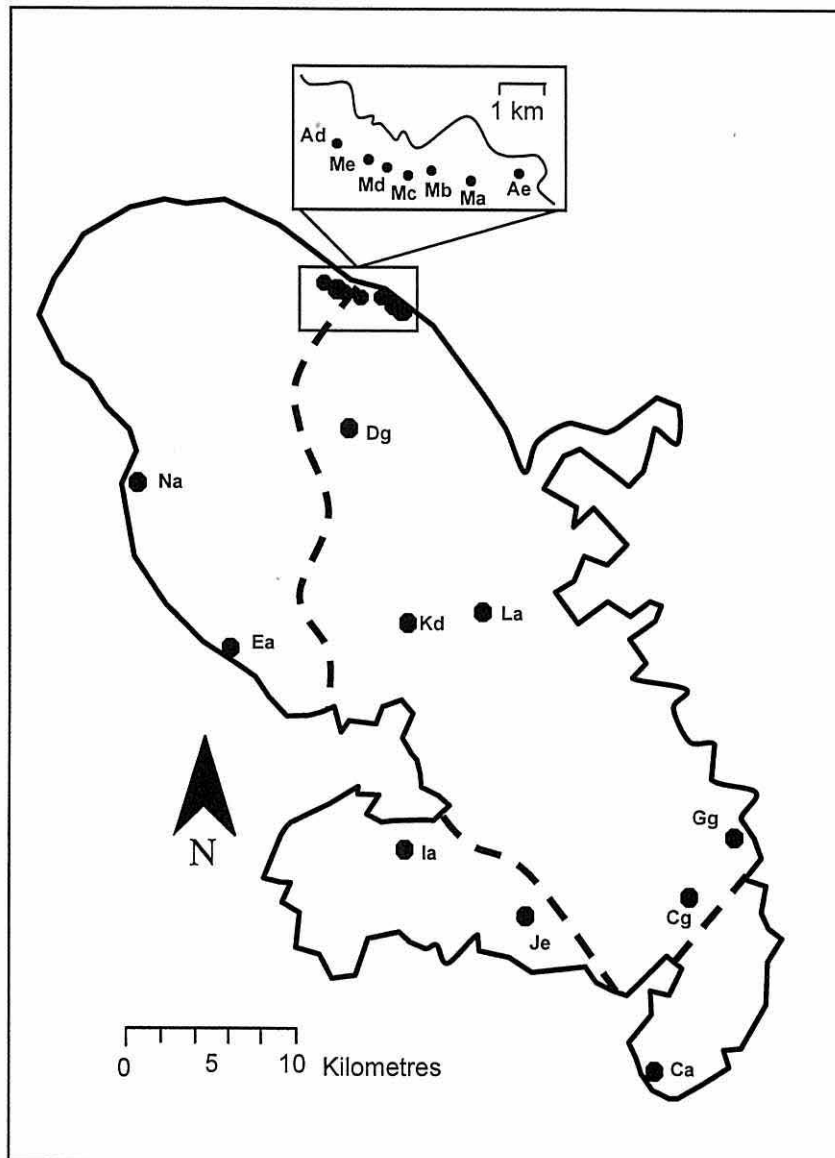
Figure 4.1: Map of Martinique, showing the island-wide sampling localities and the transect at the smaller sampling scale (inset). Broken line shows the phylogenetic lineage boundaries for the four main mtDNA lineages of *A. roquet* on Martinique

Autotomised tail tips were collected for DNA analysis and stored in 100% ethanol. Genomic DNA was extracted by the Chelex method described by Estoup et al. (1996). Individuals were typed at nine microsatellite markers (Ogden et al., 2002; Gow et al., 2006; Johansson et al., 2008a). Loci were amplified in a multiplex PCR (primer concentrations: 0.05 µM for ARO-035, ARO-062, ARO-065 and ARO-HJ2, 0.1 µM, for ARO-120 and 0.2 µM for ABO-P4A9, AAE-P2F9 and ALU-MS06) using Qiagen Multiplex PCR kit

Table 4.1: Locality names, GPS position (UTM Easting and UTM Northing), total number of genotyped samples ($N_{(T)}$), number of females ($N_{(F)}$) and number of males ($N_{(M)}$) for each locality.

Locality	UTM Easting	UTM Northing	$N_{(T)}$	$N_{(F)}$	$N_{(M)}$
Island-wide localities					
Ca	728245	1593645	28	15	13
Cg	730021	1604099	38	19	19
Dg	709480	1632216	35	17	18
Ea	702159	1618280	48	25	23
Gg	732909	1607713	32	14	18
Ia	712794	1606954	41	22	19
Je	720504	1603147	34	17	17
Kd	713197	1621052	43	19	24
La	717409	1621378	38	19	19
Na	696124	1629433	35	17	·18
Transect localities					
Ad	708108	1640748	40	20	20
Ae	711603	1640234	36	18	18
Ma	710657	1640090	40	20	20
Mb	709933	1640253	44	22	22
Mc	709442	1640153	38	19	19
Md	709089	1640332	40	20	20
Me	708722	1640418	43	22	21

following the manufacturers instructions, with the exception of an annealing temperature of 55°C. The amplified products were then analysed on an ABI 3130*xl* genetic analyser with the internal size standard 600-LIZ, and genotypes scored using GENEMAPPER v4.0 (Applied Biosystems).

From the ten localities sampled island-wide, a total of 372 individuals were genotyped, ranging from 28-48 individuals per population (mean 39.5), of which 184 were female and 188 were male. The proportions of sexes genotyped from each locality can be found in Table 4.1. From the transect, a total of 281 individuals were genotyped, with 36-44 individuals per locality (mean 40.1), totalling 140 females and 141 males (Table 4.1).

Genetic data from the two sets of localities were analysed separately. The software AR-LEQUIN v.3.01 (Schneider et al. (2000): http://lgb.unige.ch/arlequin/) was used for exact testing of Hardy-Weinberg equilibrium (Guo and Thompson, 1992) and calculation of

linkage disequilibrium (Slatkin and Excoffier, 1996) for each population and locus. Departure from Hardy-Weinberg equilibrium was considered with, and without, Bonferroni correction. For all calculations of F-statistics we used Weir and Cockerham's (1984) unbiased estimators. To test for overall genetic differentiation in the two samples and for pairwise differentiation between individual populations, we used FSTAT v.2.9.3 (Goudet (1995): http://www2.unil.ch/popgen/softwares/fstat.htm) to calculate global and pairwise F_{ST} respectively.

To test for sex-biased instantaneous dispersal we calculated the gene diversity (H_S), F_{IS}, F_{ST}, mean assignment index (mAIC) and variance of the assignment index (vAIC) separately for each sex. Statistical significance for these indices was determined by 10,000 randomizations as implemented in FSTAT v.2.9.3. A bias in dispersal between the sexes should be reflected in statistically significant dissimilarity in the estimated parameters. The higher-dispersing sex should have a higher F_{IS}: in the dispersing sex individuals sampled from one single patch will be a combination of residents and immigrants, hence a heterozygote deficit is expected due to the Wahlund effect (Goudet et al., 2002). More similar allele frequencies are expected between sampling sites in the dispersing sex and less differentiation between populations. Thus F_{ST} is expected to be higher in the philopatric sex and within-site gene diversity (H_S) is expected to be lower (Goudet et al., 2002). The measure of F_{ST} (i.e. among-population differentiation) on the island-wide sampling scale is unlikely to be informative with respect to actual dispersal rates as it is unlikely that populations exchange migrants over these distances. The probability of a genotype originating in the population from which the genotyped individual was sampled can be calculated as an assignment index, from which the sample mean (mAIC) can be found (Paetkau et al., 1995). A relatively higher frequency of rarer genotypes is expected in populations of the dispersing sex and this is indicated by a negative assignment index (Paetkau et al., 1995; Prugnolle and de Meeus, 2002). Finally, the variance of assignment indices (vAIC) can be estimated from mAIC, where variance is expected to be larger for the sex that disperses (Favre et al., 1997). In the case of microsatellite data (or other biparental markers), these methods detect only short-term dispersal, since this signal disappears after the dispersing individuals mate, due to the Mendelian segregation of biparental markers (Goudet et al., 2002).

4.4 RESULTS

The mean number of alleles scored per population (averaged over all loci) from the ten island-wide localities sampled was 10.4. There was no evidence of consistent departures from Hardy-Weinberg equilibrium or of linkage disequilibrium in the samples (Table S1 - Appendix A). The global F_{ST} was estimated at 0.059, with pairwise F_{ST} values ranging from 0.0157 to 0.1227, suggesting moderate levels of genetic differentiation on the sampled spatial scale. Males display significantly higher gene diversity, H_S (males = 0.778, females = 0.756, P = 0.005) and significantly lower mAIC values (males = -0.491, females = 0.502, P = 0.003). The remaining indices (F_{IS}, F_{ST} and vAIC) were compatible with male-biased dispersal, but did not reveal statistically significant differences (Table 4.2).

Table 4.2: Deviations from Hardy-Weinberg expectations (F_{IS}), F_{ST}, gene diversity (H_S) mean assignment index (mAIC) and variance of mean assignment index (vAIC) for the ten island-wide localities for females (F) and males (M). *P*-values are from two-tailed tests where; ** = significant at P <0.01.

	F_{IS}	F_{ST}	H_S	mAIC	vAIC
F	0.012	0.062	0.756	0.502	9.719
M	0.022	0.057	0.778	-0.491	11.533
P-value	0.597	0.404	0.005**	0.003**	0.406

The mean number of alleles scored per population on the transect was 10.62. No consistent departures from Hardy-Weinberg equilibrium and no linkage disequilibrium were detected. The global F_{ST} was estimated at 0.011, with pairwise F_{ST} values ranging from 0.0024-0.0213, suggesting low levels of genetic differentiation. Four tests of sex-biased dispersal were found to be significant on this sampling scale. Females had a negative F_{IS} (0.009) compared to the positive value for males (0.044), showing a highly significant difference (P = 0.003). The variance of assignment index also showed a significant difference (P = 0.014), with males having a higher index (13.574) compared to females (9.211). Females also showed significantly higher differentiation compared to males (female F_{ST} = 0.014, male F_{ST} = 0.006, P = 0.025). Furthermore, males and females differed significantly in mAIC, with males returning a negative value of 0.347, while females showed a positive value of 0.345 (P = 0.047). The difference in gene diversity was not significant, although the level in males was on average higher (Table 4.3).

Table 4.3: Deviations from Hardy-Weinberg expectations (F_{IS}), F_{ST}, gene diversity (H_S) mean assignment index (mAIC) and variance of mean assignment index (vAIC) for the seven transect localities for females (F) and males (M). *P*-values are from two-tailed tests where; ** = significant at $P < 0.01$, * = significant at $P < 0.05$.

	F_{IS}	F_{ST}	H_S	mAIC	vAIC
F	-0.009	0.014	0.791	0.345	9.211
M	0.044	0.006	0.795	-0.347	13.574
P-value	0.003**	0.025*	0.301	0.047*	0.014*

4.5 DISCUSSION

We include island-wide sampling (covering lineages and habitats) so that, in spite of limitations, we can generalise some our findings to the entire species. The distances between the island-wide populations are well beyond the distance juvenile anoles are expected to disperse, on account of their small size. Furthermore, the ten island-wide localities were sampled during different years. These two factors mean that among-population indices (pairwise F_{ST}'s) cannot be used to infer sex-biased dispersal at this scale (Goudet et al., 2002), but the within-population indices are informative as they are not affected by the sampling protocol. The transect localities were sampled in the same year and in the same habitat, and given the short distance between the localities, pairwise comparisons are expected to be reliable.

All of the indices estimated, on both spatial sampling scales, suggest that males have a higher dispersal rate than females in *Anolis roquet*. Highly significant differences between males and females were observed in the estimates of mean assignment index and gene diversity on the island-wide sampling scale, whereas F_{IS}, F_{ST}, mAIC and vAIC showed significant differences on the transect. The evaluation by Goudet et al. (2002) of the efficiency and power of these tests showed that test performance was dependent on dispersal rates, strength of bias, polymorphism of the markers and the sampling. These tests generally have a low power in detecting bias, unless the sex-bias in dispersal is at least 80:20 and all populations involved are sampled exhaustively (Goudet et al., 2002). Within this context, the significant indices detected in this study provide strong evidence for male-biased dispersal in this species. The results for *A. roquet* are in concordance with the prediction of male-biased dispersal in polygynous species, which was first suggested by Greenwood (1980). However, not all polygynous lizards show male-biased dispersal (Olsson et al., 1996; Olsson and Shine, 2003), and several reasons can be postulated for the results observed in *A. roquet*.

It has been shown that in cases where males compete for females (local mate competition) and females compete for resources (local resource competition), the association between male-biased dispersal and polygyny is strengthened (Perrin and Mazalov, 2000). Similarly, higher levels of competition between males than between females in a polygynous system increase the likelihood of a male-bias in dispersal. This occurs in the female defence polygyny observed for sexually dimorphic members of *Anolis* (Trivers, 1976; Schoener and Schoener, 1980; Jenssen et al., 2001). Female anoles choose their territory before they reach sexual maturity and several females can hold home ranges that overlap considerably (Trivers, 1976; Jenssen et al., 2001). Males subsequently enter female territory and dominant males defend their access to, and mate with, several females (Schoener and Schoener, 1980; Jenssen et al., 2001). Therefore, females may benefit from dispersing only to the extent to which they are able to obtain adequate resources for survival and reproduction (Stamps, 1977), whereas males may have to disperse further to find territory that is unoccupied by superior males. In those anoles that have polygynous mating systems it appears that intrasexual aggression levels are consistently higher in males than in females (Trivers, 1976; Jenssen et al., 2000), and this aggression has been hypothesised to cause juvenile or sub-adult males to leave their natal area (Trivers, 1976; Schoener and Schoener, 1980, 1982).

Conversely, philopatry is typically favourable for the sex that invests highly in their offspring, given that knowledge of the territory and potential social interactions with kin may provide benefits (Greenwood, 1980; Waser and Jones, 1983). In anoles, as in most species of lizard, there is no post-hatchling parental investment, however females invest more energy in reproduction (for egg production) than males (Orrell et al., 2004) suggesting benefits for philopatric females. As described above, there is a very strong mtDNA structure in *A. roquet*. Specific lineages are associated with the different precursor islands, even after an estimated 1.5 million years of secondary contact and in the absence of physical or ecological barriers (Ogden and Thorpe, 2002; Thorpe et al., 2008). Such a pattern has been described in several species (Taberlet and Bouvet, 1994; Gibbs et al., 2000; Waits et al., 2000; Castella et al., 2001; Petit et al., 2001) and has been explained by very high philopatry in females. A comprehensive study of this type of pattern comes from the greater mouse-eared bat *Myotis myotis* (Castella et al., 2001). In this species females aggregate to form nursing colonies in spring and summer, and the females have been shown to exhibit strong fidelity to their natal colonies, whereas the geographic origin of males is usually unknown. Castella et al. (2001) show that dispersal of effectively a single sex in *M. myotis* is sufficient to homogenise nDNA structure whilst preserving more mtDNA structure, when the other sex is philopatric. Similar conclusions were drawn by

Bowen et al. (2005), in a study on loggerhead turtles. Hence the structure observed in *A. roquet* mtDNA is probably due to strong female philopatry, as observed previously in *Anolis oculatus* by Stenson et al. (2002).

In species with strong philopatric tendencies, sex-biased dispersal is beneficial since it decreases the risk of mating with related individuals (Pusey, 1987). Furthermore, the potential cost of inbreeding associated with philopatry may also be reduced by multiple paternity in anoles. Some female anoles have been shown to be highly promiscuous, and able to store sperm for more than two months (Fox, 1963; Calsbeek et al., 2007; Eales et al., 2008). Offspring of promiscuous females are genetically more diverse, thereby allowing maternal half-siblings to mate with a decreased risk of inbreeding (Calsbeek et al., 2007).

In conclusion, this study shows strong evidence for male-biased dispersal in a species for which direct dispersal estimation by mark-recapture methods would be very difficult to obtain. This result contributes to an explanation of the conflicting patterns of gene flow between nuclear and mitochondrial markers observed by Thorpe et al. (2008). Indeed, it seems likely that pronounced female philopatry produced the strong mtDNA lineage structure observed across the island (Thorpe and Stenson, 2003), while nuclear gene flow across the lineage contact zones may be predominantly maintained by means of male dispersal.

THE ROLES OF ALLOPATRIC DIVERGENCE AND NATURAL SELECTION IN QUANTITATIVE TRAIT VARIATION ACROSS A SECONDARY CONTACT ZONE IN THE LIZARD *Anolis roquet*

5.1 ABSTRACT

Populations of the Caribbean lizard, *Anolis roquet*, are thought to have experienced long periods of allopatry before recent secondary contact. To elucidate the effects of past allopatry on population divergence in *A. roquet*, we surveyed parallel transects across a secondary contact zone in northeastern Martinique. We used diagnostic molecular mtDNA markers to test fine-scale association of mtDNA lineage and geological region, multivariate statistical techniques to explore quantitative trait pattern, and cline fitting techniques to model trait variation across the zone of secondary contact. We found that lineages were strongly associated with geological regions along both transects, but quantitative trait patterns were remarkably different. Patterns of morphological and mtDNA variation were consistent with a strong barrier to gene flow on the coast, whereas there were no indications of barriers to gene flow in the transitional forest. Hence, the coastal populations behaved as would be predicted by an allopatric model of divergence in this species complex, while those in the transitional forest did not, in spite of the close proximity of the transects and their shared geological history. Patterns of geographic variation in this complex, together with environmental data, suggest that on balance, selection regimes on either side of the secondary contact zone in the transitional forest may be more convergent, while those either side of the secondary contact on the coast are more divergent. Hence, the evolutionary consequences of allopatry may be strongly influenced by local natural selection regimes.

5.2 INTRODUCTION

Hybrid zones are areas where distinct genotypes or phenotypes meet and produce hybrids (Barton and Hewitt, 1985), and as such they are relevant to studies of speciation (Harrison, 1991; Nurnberger et al., 1995; Jiggins and Mallet, 2000). Hybrid zones arise from either primary or secondary contact (Barton and Hewitt, 1985). In the latter case, differences built up during a period of geographic isolation are effectively put to a test of compatibility (Fitpatrick and Shaffer, 2004). On initial contact, steep and congruent clines in multiple characters may form. With time, and in the absence of strong selection, these clines can disappear due to the homogenising effects of dispersal and recombination (Endler, 1977; Barton and Hewitt, 1985), and previously isolated populations may merge (Sequeira et al., 2004). Alternatively, the centre and width of some clines may displace and flatten (respectively) in response to varying selection pressures on different traits (Parsons et al., 1993; Nurnberger et al., 1995; Brumfield et al., 2001; Takami and Suzuki, 2005). Finally, multiple character clines may remain steep and congruent in response to strong selection against hybrids (Dasmahapatra et al., 2002; Phillips et al., 2004), offering the possibility of continued population divergence.

Estimating the relative contribution of two types of selection, endogenous (intrinsic) and exogenous (extrinsic), is a fundamental problem in hybrid zone study (Kruuk et al., 1999). Endogenous selection leads to the formation of tension zones, which are independent from the environment and predominantly maintained by reduced fitness in hybrids due to an incompatible mix of genomes (Barton and Hewitt, 1985; Nurnberger et al., 1995; Phillips et al., 2004). Exogenous selection results in hybrid zones where different types may be favoured on either side of an environmental gradient, and it is the environmental gradient that ultimately determines the position of the cline (Nurnberger et al., 1995). In patchy environments, exogenous selection can lead to the formation of mosaic hybrid zones. In these zones, character traits show abrupt reversals and transitions in concordance with habitat distribution (Ross and Harrison, 2002; Vines et al., 2003; Fitpatrick and Shaffer, 2004). Endogenous and exogenous selection can act together (Szymura and Barton, 1986, 1991; Sites et al., 1995) and distinguishing the effects of one from the other can be difficult or impossible (Kruuk et al., 1999; Marshall and Sites, 2001). The relative strengths of endogenous and exogenous selection pressures can also vary in different areas of a zone of secondary contact (Hairston et al., 1992; Szymura and Barton, 1991; Vines et al., 2003) and clines can move, either as a response to change in environment (Dasmahapatra et al., 2002; Leache and Cole, 2007), due to competition (Rohwer et al., 2001) or into areas of low population density (Barton and Hewitt, 1985). The fate of clines in secondary contact

zones is ultimately concerned with fundamental theories of speciation and species concepts. Case studies from hybrid zones can offer insight into the effects of allopatry and the importance of other forces driving population divergence upon secondary contact.

Adaptive radiations, such as the *Anolis* radiation in the Caribbean, are particularly useful for the empirical study of the processes involved in speciation. The circa 150 species of anoles in the Caribbean demonstrate high levels of in-situ speciation from as few as two colonisation events (Jackman et al., 1999). The islands involved in this adaptive radiation are grouped into two sets based on island size and geology: the Greater Antilles, which include the large islands of Cuba, Hispaniola, and Puerto Rico, and the Lesser Antilles, a chain of small islands extending from Anguilla, south towards Venezuela. The anole communities on the two sets of islands share some fundamental similarities. Specifically, there is strong evidence in both areas of natural selection acting on quantitative traits. Selection has led to both intra-specific within-island adaptation to habitat in the Lesser Antilles (Thorpe and Malhotra, 1996; Malhotra and Thorpe, 2000) and divergent habitat specialization among species in the Greater Antilles (Losos, 2004; Calsbeek et al., 2006). However, the Greater Antillean islands support multi-species anole communities with up to 55 endemic species on a single island (Losos et al., 2003), whereas the Lesser Antilles have only one or two native species on each island (Thorpe et al., 2004). In the Lesser Antilles both the biogeographic pattern of species distribution and phylogenetic analyses of molecular variation suggest that anole colonization and subsequent speciation occurred progressively from island to island (Thorpe and Stenson, 2003; Thorpe et al., 2004, 2008), suggesting that allopatry is important for speciation in this particular set of islands.

Because the Lesser Antilles are a chain of discrete islands, their respective species of anole are geographically isolated from each other. Therefore, reproductive isolation in this group cannot usually be tested in natural conditions. However, the island of Martinique is unusual due to its geological history: it appears that it was formed into a single island from five separate precursor islands, when two precursors from the older arc (Caravelle, and St Anne peninsulas, formed during the Eocene and early Miocene) and two precursors from the younger arc (Trois-Ilets peninsula and the northwest, formed during the Miocene and Pleistocene) were joined by the uplifting of the central area between them, possibly as recently as 1.5 million years ago, or less (Andreieff et al., 1976; Bouysse et al., 1983; Maury et al., 1990; Sigurdsson and Carey, 1991; Thorpe et al., 2008). This has allowed organisms that evolved in allopatry to come into secondary contact, offering an exceptional opportunity in which to test the strength of allopatric divergence and its role in speciation, while also evaluating the importance of selective forces driving differ-

entiation.

Martinique is inhabited by a single species of anole, *Anolis roquet*, which is an endemic, arboreal, iguanid lizard. A recent phylogenetic analysis of a 1139 base pair mtDNA (cytochrome *b*) fragment revealed four main monophyletic lineages (Figure 5.1) (Thorpe and Stenson, 2003). Across the island, these main lineages were found to be very closely associated with the geological regions described above. The ages of the lineages directly correspond to the emergence of the most recent precursor islands and regions, and it is thought that young precursor islands were colonised as they emerged (Thorpe and Stenson, 2003). When volcanic activity and orogeny eventually connected the precursor islands to form present-day Martinique, lizard lineages came into secondary contact (Thorpe et al., 2004). Three major contact zones were identified on Martinique, where divergence between the different lineages has been estimated at between 6 and 8 million years, probably followed by less than 1.5 million years of secondary contact (Thorpe et al., 2008).

The mountainous younger arc Lesser Antillean islands, such as Martinique, have pronounced environmental zonation, with specific vegetation types (Beard, 1948). Rainforest covers the montane interiors, and is replaced by transitional forest as altitude decreases towards the coast. Coastal habitat varies, with xeric woodland in the rain shadow of the Caribbean coast, and littoral woodland on the exposed central Atlantic coast. Furthermore, rainfall and habitat change on the southern and northern tips of the islands. As a result, in Martinique the Atlantic littoral woodland is replaced by xeric woodland to the south and mesic forest to the north.

The Martinique anole, like the anoles on the other Lesser Antillean islands with environmental zonation, shows marked geographic variation in hue and pattern, as well as scalation, body dimensions and size (Lazell, 1972; Thorpe and Malhotra, 1996; Thorpe and Stenson, 2003; Thorpe et al., 2004, 2008). There may be specific exceptions, but overall, the geographic variation in quantitative traits (QT) of anoles on Martinique, and the other Lesser Antillean islands, is associated with this environmental zonation rather than phylogeographic lineages. This is interpreted as natural selection for current conditions and is supported by common garden experiments indicating genetic control rather than plasticity (Thorpe et al., 2005), large scale field experiments on natural selection determining the intensity and targets of selection (Malhotra and Thorpe, 1991; Thorpe et al., 2005), correlations between environmental and QT patterns (taking into account phylogeny and other factors), and parallel patterns of adaptation (Thorpe, 2005). The latter is particularly notable in the Martinique anole where populations from different lineages experiencing
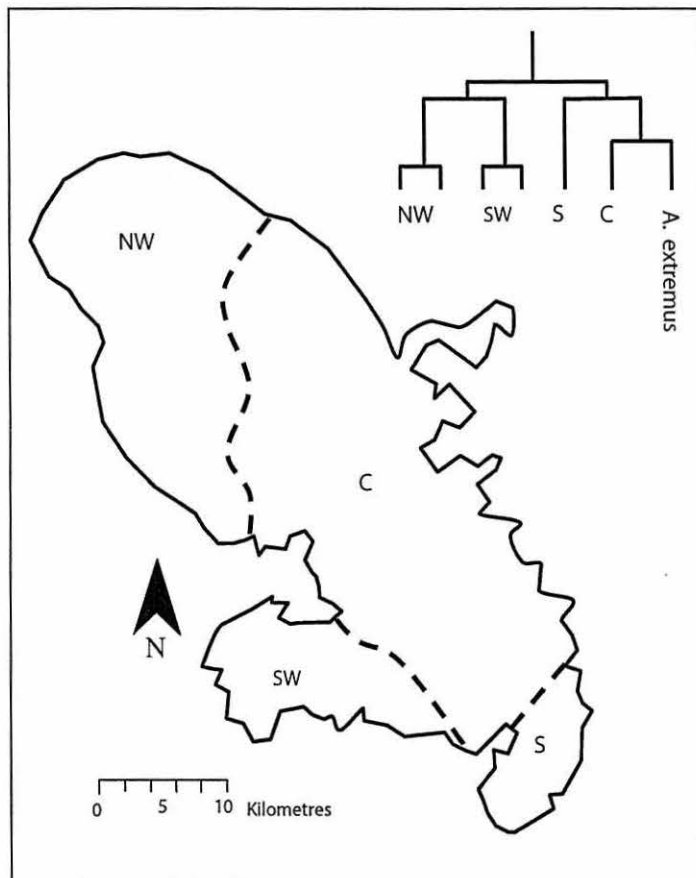
Figure 5.1: Main phylogenetic relationships and lineage boundaries of *A. roquet* on Martinique (Thorpe and Stenson, 2003; Thorpe et al., 2008). *Anolis extremus* on Barbados is a sister clade to the central *A. roquet lineage* (Thorpe and Stenson, 2003).

similar environmental conditions have very similar appearance due to strong convergent selection (Thorpe, 2005). An example of this is found in montane forms which, irrespective of lineage, are an intense saturated green hue with black and non-UV white markings. Indeed, Ogden and Thorpe (2002) and Thorpe et al. (2008) show that where northwestern and central lineages meet in montane rainforest after prolonged allopatry, convergent selection renders them identical (in patterns of QTs) and there is no indication of reduction in gene flow across the secondary contact zone as estimated by neutral nuclear markers. In sharp contrast, divergent selection between adjacent habitats results in marked difference in QTs and a notable reduction in gene flow estimated by the same nuclear markers (Ogden and Thorpe, 2002; Thorpe et al., 2008). Despite this general pattern, Thorpe and Stenson (2003) identified two areas where, at least superficially, quantitative trait variation seems to correlate to lineage, one in southern Martinique (Ste Anne peninsula) and

the other on the north Atlantic coast.

This study examines one of the regions that may represent an exception to the general pattern. In north-eastern Martinique, two of the most divergent mtDNA lineages meet (central and north-western, 7.9 %uncorrected divergence). Where these lineages meet on the coast, the Atlantic littoral woodland (Beard, 1948) gives way to wetter and more seasonal climatic conditions to the north. However, inland at the transitional forest, conditions are more consistent at any given altitude, and in the montane rainforest there is thought to be strong convergent selection for identical habitats either side of the secondary contact between these two lineages. Along the coastal strip of littoral woodland, anoles from the central lineage (C) have a brown, dull, uniform dorsum with a low UV reflective dewlap, whereas anoles from the north-western (NW) lineage are green with black markings and a bright dewlap in the yellow/orange part of the spectrum and higher UV reflection (Figure 5.2). Colour and pattern appear correlated with lineage, and lineage forms are easily distinguished on the coast over the space of a few kilometres. The change in dewlap hue along the Atlantic coast of Martinique (Thorpe and Stenson, 2003) may be in response to habitat change, as *A. trinitatis* on the Atlantic coast of St Vincent also has a highly UV reflective dewlap (Thorpe, 2002). However, in the transitional forest, a short distance towards the interior of the island, lizards from both lineages are superficially indistinguishable (Figure 5.2).

With two parallel transects, one on the coast and one in the transitional forest, we explore the transition between mtDNA lineages and relate these to quantitative traits known to be under selection (colour, body dimensions, markings and scale counts, (Ogden and Thorpe, 2002; Malhotra and Thorpe, 1991; Thorpe, 2005). We also measure climatic and habitat data along the transects in order to give an insight into the nature of selection (convergent or divergent) influencing populations on either side of the contact zone. The estimation of the position of mtDNA and quantitative traits cline centres, and their respective widths, allows us to gain insight into the formation and maintenance of the secondary contact zones (Leache and Cole, 2007). The influence of allopatry and natural selection on quantitative traits variation also is discussed.
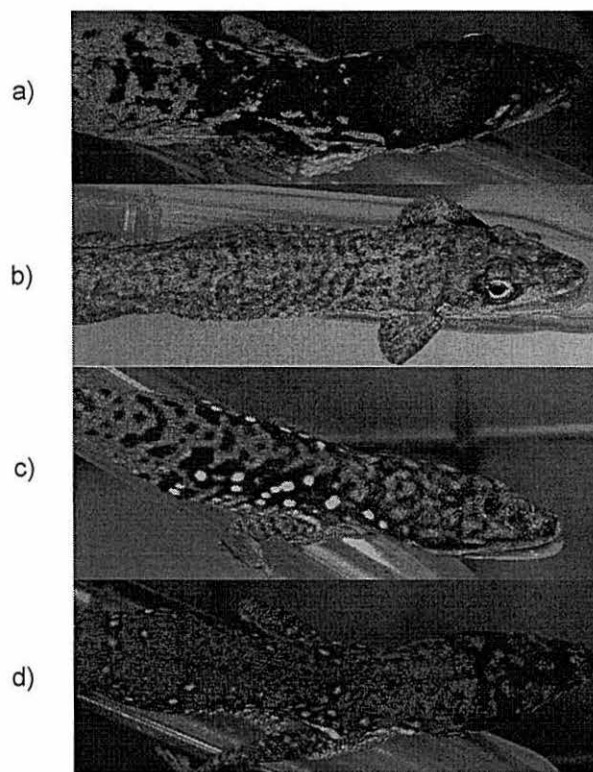
Figure 5.2: Marking patterns in *A. roquet* from north-eastern Martinique. a) Northern coastal form (north-western lineage) with black head, black cloak and lightspots on a green background. b) Southern coastal form (central lineage). Black markings and lightspots are entirely absent and lizards are brown. c)-d) Typical lizards from transitional forest habitat (both lineages); black markings and light spots are present to varying degrees, background colour is green.

5.3 METHODS

5.3.1 SAMPLING

Eight localities along the coast and eight localities in transitional forest habitat were sampled along two linear parallel transects approximately 15 km in length (Figure 5.3). The average distance between transects was approximately 6 km. Transects were designed to traverse the boundary between NW and C lineages of *Anolis roquet* as described by Thorpe and Stenson (2003). Forty-eight lizards from each locality were hand caught and sampled for DNA (autotomised tail tips). Tail tips were stored in tubes containing absolute ethanol for subsequent genetic analysis.
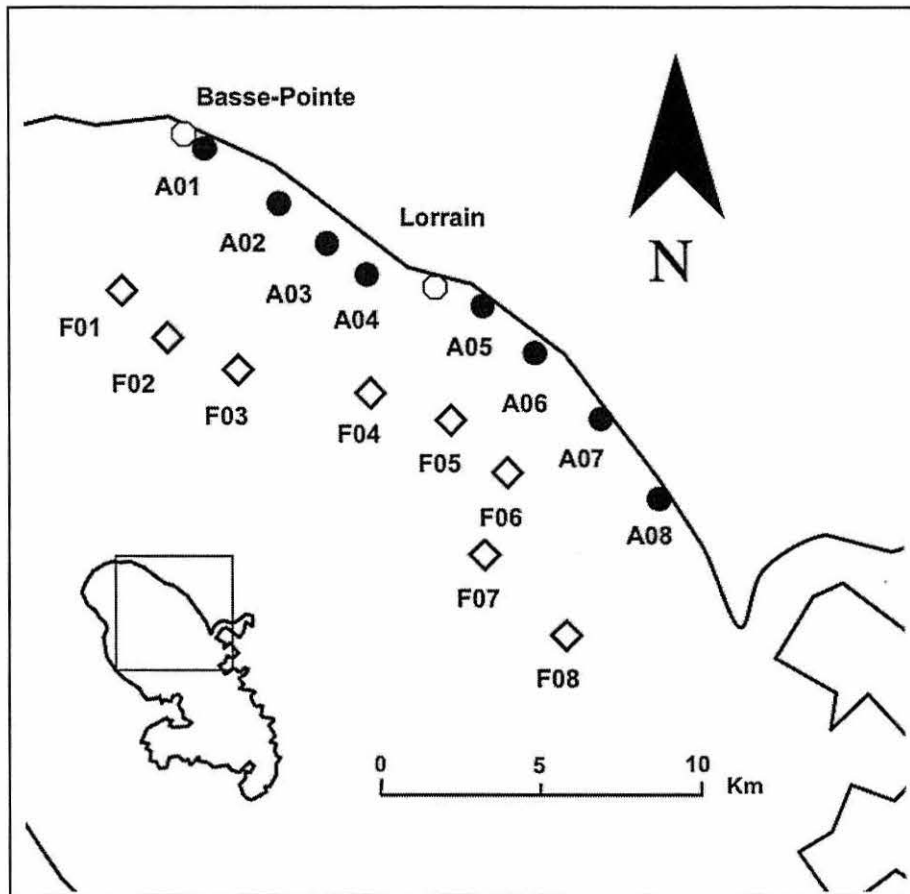
Figure 5.3:   Map showing the position of the transects. The townships of Basse-Pointe and Lorrain are included for geographical reference, and are represented by unfilled circles. Inset shows the island of Martinique; study area is boxed.

5.3.2 DIAGNOSTIC PCR-RFLP ASSIGNMENT

We designed a PCR-RFLP assay to quickly and confidently assign individuals to their mitochondrial clade without the need to sequence them. To do this, we aligned all the cytochrome b sequences published by Thorpe and Stenson (2003) and examined them for fixed differences between clades that corresponded to restriction sites. The restriction enzyme SspI was found to cut the cytochrome b fragment at position 160 in the NW clade, but not in the C clade, and was subsequently used to distinguish the two clades. A total of 765 DNA samples were extracted from collected tail tips using Qiagen DNeasy Blood & Tissue Kit (Qiagen). A 1063 basepair fragment of the cytochrome b gene was amplified using the primers MtA-S (5'-ATCTCAGCATGATGAAACTTCG-'3) and MtF-S (5'-TTTGGTTTACAAGACCAATG-'3) in 10 μl reactions using 5 ng of template DNA, 3 mM MgCl$_2$, 0.1 mM of each nucleotide, 0.4 μM of each primer, 0.5 units of Taq DNA

polymerase (Promega), and 10x buffer (50 mM KCL; 10 mM Tris-HCl, pH 9.0). PCR reactions were performed using a profile of denaturation of 5 min at 95 °C, followed by 34 cycles (20 s for 95 °C, 30 s at 48 °C, and 45 s at 72 °C) with a final extension period of 5 min at 72°C. After amplification, a mixture of 0.2 µl *Ssp*I (New England Biolabs), 2 µl buffer 2 (NEB) and 7.8 µl H2O was added and the samples were digested for 3 hours at 37 °C. The reaction was stopped by denaturation of the enzyme at 85 °C for 15 minutes. Products were visualised on 1.5 % agarose gels stained with ethidium bromide under UV illumination, and scored from photographs.

5.3.3 QUANTITATIVE TRAIT VARIATION

A subset of ten adult males from each locality were subject to quantitative trait analysis of the following 20 characters from four character sets (see Table 2.1 and Figure 2.1): (1) body dimensions: snout to vent length (SVL) jaw length (JL), head length (HL), head depth (HD), head width (HW), upper hind leg length (ULL), lower hind leg length (LLL), dewlap length (DL), (2) scale counts: postmental (PSC), supraorbital semicircle (SSC), dorsal (DSC) and ventral (VSC), (3) markings: number of light patches on head (LPH), number of light patches on anterior body (LPA), number of light patches on posterior body (LPB), percentage of black hood covering head (HEAD), percentage of black cloak covering anterior body (CLOAK). Prior to recording body measurements and marking patterns, lizards were photographed in standardized light conditions with a Canon EOS 350D fitted with a 100mm Canon macro lens and a Macro Twin Lite MT-24EX flash. Body dimension measurements were taken in mm using electronic digital callipers (Linear Tools, U.K.), accurate to two decimal points. Photographs were used to confirm scoring of marking patterns, to perform the ventral and dorsal scale counts and were also manipulated in Photoshop (Adobe Systems, U.S.A.) to extract the fourth character set; a measure of hue based on the relative proportion of green, red and blue pixels within a standardized area on the dorsal trunk, just behind the front legs. Earlier spectrophotometric analysis of populations in this area (Thorpe and Stenson, 2003) indicated that this was an acceptable procedure for dealing with the hue variation of this part of the body when using large samples.

Quantitative traits were analysed independently for each transect. Unless stated, variables were normally distributed. Body dimensions were adjusted against snout-vent length by analysis of covariance (ANCOVA). For each transect, five categorical markings data and two scale counts (SSC and PSC) that had distributions that violate the assumptions

of canonical variate analysis (CVA) were entered into a principal components analysis (PCA). The seven components did not violate these assumptions and were input into subsequent CVAs as in Dunteman (1989); Zhao et al. (1998); Macedonia (2001); Weisrock et al. (2005), and Stein and Uy (2007). Hence we input the seven principal components, adjusted body dimensions, two raw scale count variables (DSC and VSC) and two hue variables (red and green) into a canonical variate analysis using SPSS v.14, to study the variation along the transects.

5.3.4 Climate data

The climate in the northeast Martinique is characterised by moist trade winds blowing in from the Atlantic Ocean and reaching the slopes of the Pitons du Carbet and Mt Pele a few kilometres inland. Climate changes rapidly with increasing elevation towards the interior of the island with increased precipitation and lower temperatures in the mountains. Conditions also change with latitude towards the northern and southern extremities of the island. A large body of evidence (see above) indicates that Lesser Antillean anoles adapt by natural selection to this environmental zonation. To elucidate how environmental conditions, and hence selection regimes, varied along the transects we carried out a PCA (normalized data) on altitude (Institut Gographique National, Carte de Randone 4502 MT and 4503 MT (French national 1:25000 resolution maps)), habitat type (Lassere, 1979), and three climatic variables (annual mean temperature, annual precipitation and precipitation seasonality data) from http://www.worldclim.com. Habitat types were given a nominal code for the analysis. For both transects, correlations were performed between the first and second principal components and spatial position of the localities along the transect.

5.3.5 Cline fitting

We fitted tanH clines to mtDNA frequency and quantitative trait data using the Fit 1D cline in the program ANALYSE 1.3 (Barton and Baird (1999): http://www.biology.ed.ac.uk/ research/institutes/ evolution/software/Mac/Analyse/Version1.3.html.). The program fits tanH curves to cline data using four variables: cline width, cline centre, and P_{min} - P_{max} (where P_{min} and P_{max} denote minimum and maximum gene frequencies in the tail end of a cline), using a Metropolis algorithm. Normally distributed phenotypic data can be fitted to clines either as single characters or from composite variables (e.g. PCA or CVA scores)

where P_{min} and P_{max} are the maximum and minimum character scores in the tails of the cline (Bridle et al., 2001; Brumfield et al., 2001; Dasmahapatra et al., 2002; Takami and Suzuki, 2005; Leache and Cole, 2007).

Model parameters for mtDNA and quantitative trait clines were estimated independently for both transects, allowing centre and width to vary, fixing P_{min} and P_{max} at 0 and 1 respectively over 2000 iterations along a best-fit axis. CV 1 locality means from the quantitative trait CVA were transformed to a 0-1 scale prior to cline fitting, and mtDNA data were represented as 0-1 haplotype frequencies at each locality (Takami and Suzuki, 2005). Support values were generated using the support values option in ANALYSE 1.3, where parameter values within 2 likelihood units were generated, equivalent to 95% confidence limits. Significant coincidence and concordance between mtDNA and quantitative traits was attained if centre and width values from one cline could be found within the support limits of the other cline, corresponding to 95% confidence limits (Takami and Suzuki, 2005).

5.4 RESULTS

Group means and variances for each trait and locality can be found in (supplementary) Tables S1 and S2 (Appendix B); correlations between characters for each transect can be found in Tables S3 and S4 (Appendix B).

5.4.1 COASTAL TRANSECT

On the coastal transect the haplotype frequency change shown by the mtDNA PCR-RFLPs was a sharp step; at each extreme locality there was 100% of NW or C haplotype, a further three localities contained only C haplotypes, and in the remaining four localities more than 90% of individuals were of the common haplotype (Figure 5.4a). The changeover in quantitative traits mirrored that of the mtDNA, and the total change between extreme localities amounted to 5.5 pooled within-groups standard deviations (Figure 5.4b). There was no overlap of CV scores between the two sets of localities (A01-A04, and A05-A08), and there was a strong and significant correlation between quantitative trait locality means and lineage ($r = 0.99$, $P < 0.01$, $N = 8$). Lizards from either lineage were thereby clearly separated by their appearance: lizards belonging to the north-western lineage were green with black markings and light-patches, this contrasted with lizards be-

longing to the central lineage which were brown, without black markings or light-patches, and with relatively wider heads (Tables S5 and S6 - Appendix B). The bioclimatic PC2 indicated a significant linear change in likely selection regime along transect A ($r = 0.92$, $P <0.001$, $N = 8$) (Table S7 - Appendix B), where the south is less seasonal and habitat is dominated by xerophilous vegetation, although PC1 did not change along the transect ($r = 0.17$, $P >0.68$, $N = 8$, Figure S1 - Appendix B). There was also a strong significant correlation between QT pattern and bioclimatic PC2 variation ($r = -0.84$, $P <0.01$, $N = 8$). Furthermore, both mtDNA and QT clines were narrow with sharp transitions between locality A04 and A05, coinciding with a geological boundary (Figure 5.6a). Widths and centres coincided and concurred (Table 5.1).

Table 5.1: Cline widths and centre positions (metres from transect starts) are given for both transects, with best log likelihood for the estimations. Support limits are in parenthesis.

	Width (m)	Centre (m)	log likelihood
Coastal			
mtDNA	3016 (2549-3587)	6715 (6344-7155)	-16.304
QT	2990 (2057-4044)	6450 (6193-6767)	-0.37
Transitional forest			
mtDNA	4000 (3171-4893)	5656 (5248-6052)	-2.779
QT	13122 (8868-20518)	9834 (8444-11210)	-12.627

5.4.2 TRANSITIONAL FOREST TRANSECT

In the transitional forest transect mtDNA haplotype frequency change was gradual, with pure NW and C lineage at the extreme localities (Figure 5.5a). The changeover occurred over four localities in the northern part of the transect, and the three southernmost localities were pure C lineage. The quantitative traits showed some variation across the transect; there was a minor gradient between the first and last four localities with 2.2 pooled within-group standard deviations of change between extreme localities (Figure 5.5b). In contrast to the coastal transect, QT scores overlapped extensively so no distinct sets could be recognized. However, there was a significant correlation between QT and lineage ($r = 0.79$, $P <0.02$, $N = 8$). Here, extreme northern localities were populated with bright green lizards with black markings, while greenness and markings became less prominent further south and head depths increased (Tables S8 and S9 - Appendix B). Neither bioclimatic PC1 or PC2 (Table S10, Figure S2 - Appendix B) changed significantly along transect F (PC1 $r = -0.43$, $P = 0.29$, $N = 8$: PC2 $r = 0.63$ $P = 0.09$, $N = 8$) and we found

a) Lineage

b) Quantitative Traits
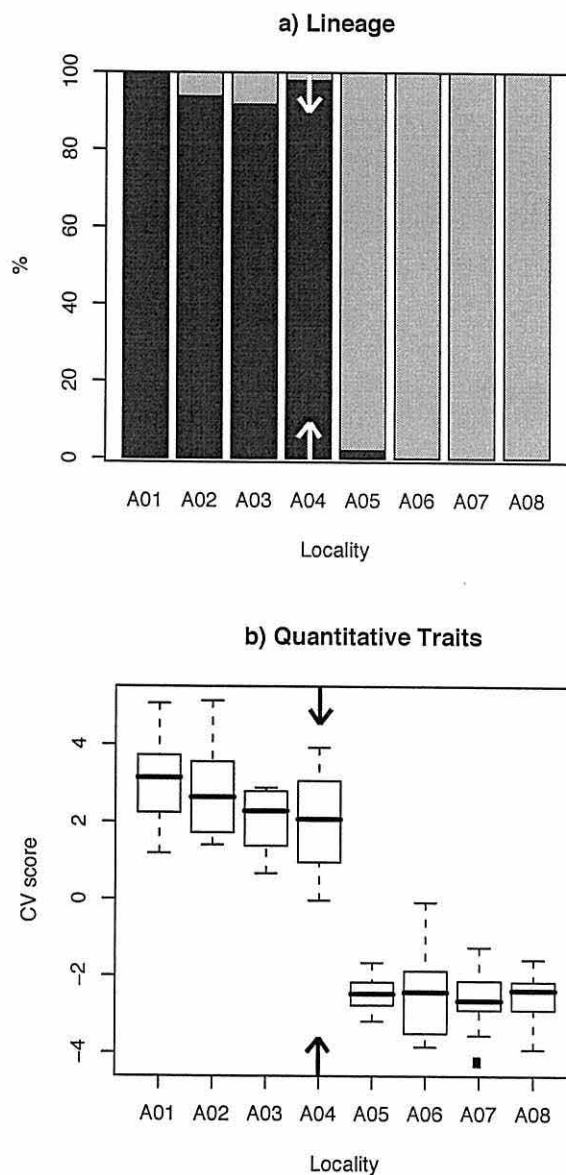
Figure 5.4: Lineage distribution and quantitative trait pattern along the coastal transect. (a) Lineage distribution. Dark grey represents north-western lineage, and pale grey represent central lineage. (b) CV scores for quantitative traits. Haplotype and quantitative trait pattern are similar with sharp, stepped transitions at the geographical boundary (boundary is indicated by arrows).

no significant correlation between QT pattern and bioclimate (PC1 $r = -0.33$, $P = 0.43$, $N = 8$: PC2 $r = 0.63$, $P = 0.10$, $N = 8$).

Quantitative trait and mtDNA clines had very different centre and widths (Table 5.1). The QT cline was nearly twice as wide as the mtDNA cline (Figure 5.6b). The centre of the mtDNA cline was located between locality F02 and F03, and therefore coincided with the

geological boundary, whereas the centre of the QT cline was displaced south.
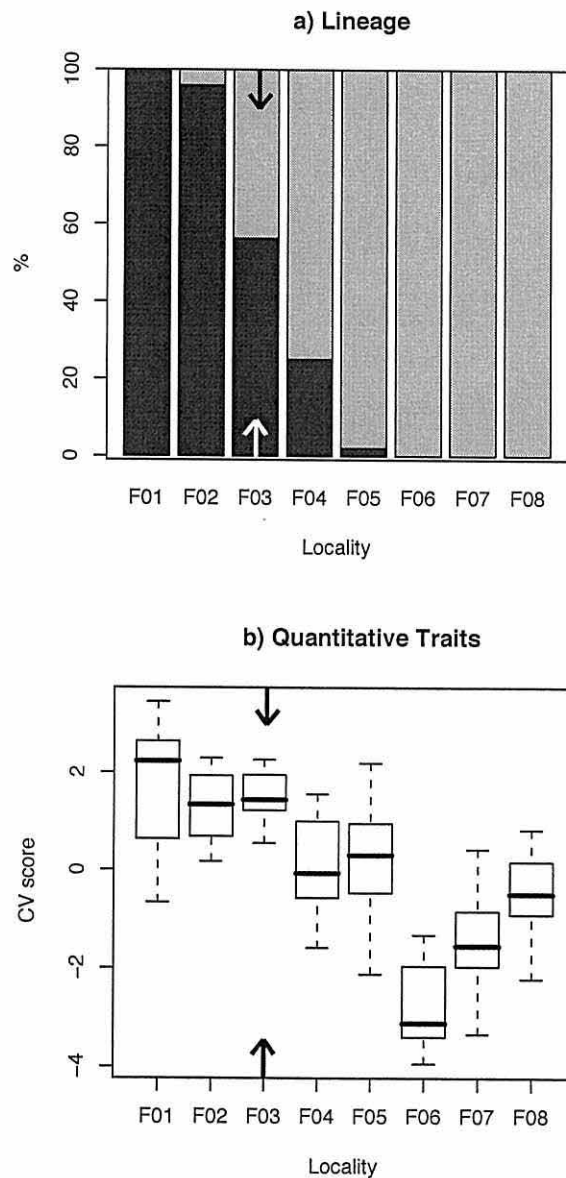
Figure 5.5: Lineage distribution and quantitative trait pattern along the transitional forest transect. (a) Lineage distribution. Dark grey represents north-western lineage, and pale grey represent central lineage. (b) CV scores for quantitative traits. Lineage and quantitative trait variation do not show similar patterns; lineage transition is gradual but coincides with geological boundary (indicated by arrows). Quantitative trait pattern show variation that does not appear to coincide with lineage transition or geological boundary.
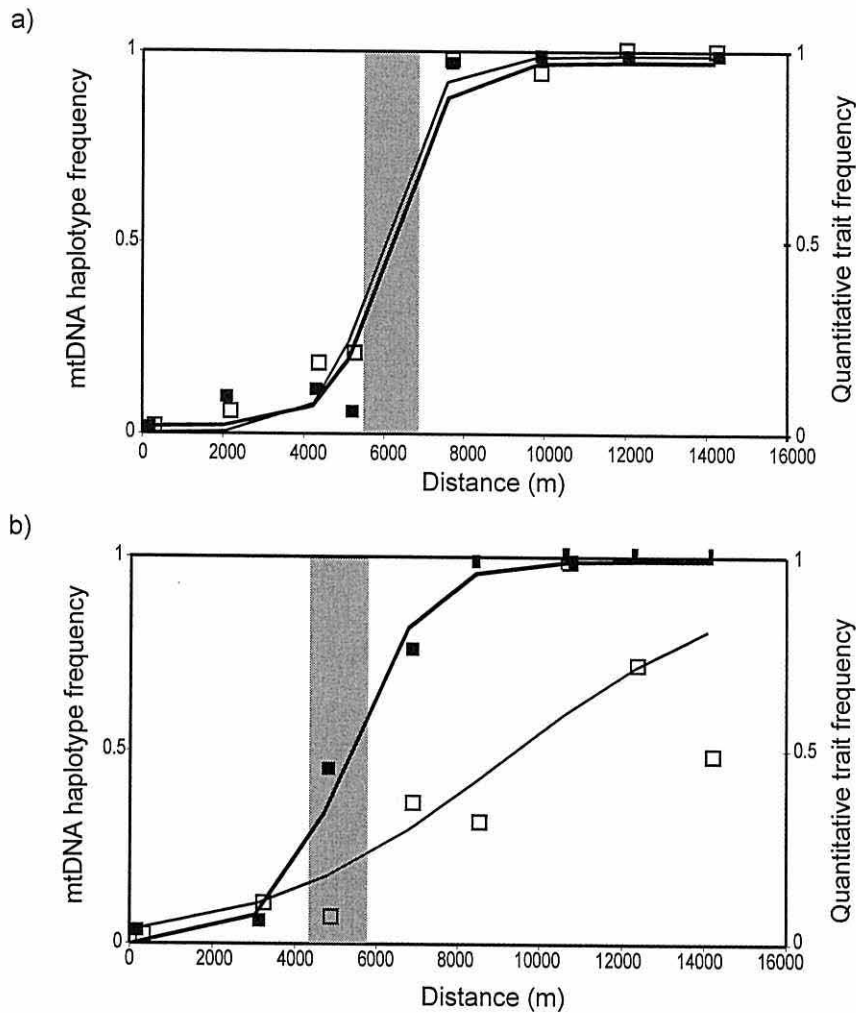
Figure 5.6: Mitochondrial DNA and quantitative traits clines. Closed squares and thick line represent mtDNA. Open squares and thin line represent quantitative traits. Geological boundary (see discussion) indicated with grey shading. Distance was measured from locality A01 on the coastal transect, and F01 on the transitional forest transect, and projected distance between localities was used. Mitochondrial DNA and quantitative traits clines concur and coincide on the coast (a) but not on the transitional forest transect (b).

5.5 DISCUSSION

Geological events corresponding with phylogenetic data show evidence of secondary contact of distinct *A. roquet* mtDNA lineages on Martinique (Thorpe and Stenson, 2003; Thorpe et al., 2008). Here we confirmed this relationship on a finer scale and with larger sample numbers; mtDNA transition examined in both transects (identified by the

mtDNA cline centre) occurred at the geological boundary where the substrate composition changes from calc-alcali to andesite volcanic rock (Andreieff et al., 1976; Maury et al., 1990; Sigurdsson and Carey, 1991). Broadly speaking, the north-western precursor island is likely to have joined the central precursor island relatively recently (1 -1.5 mybp), after their respective anole populations had spent an extended period (6-8mybp) in allopatry (Thorpe et al., 2008). However, more recent volcanic activity of Mt Pele (500k-5kybp) (Traineau et al., 1989) may have caused episodes of local extinction and recolonisation. As the transect in the transitional forest was between Mt Pele and the coastal transect it is most unlikely that this volcanism could have impacted the coastal region without simultaneously impacting the adjacent transitional forest region, so the timing of secondary contact should be the same in both transects.

Nevertheless, there were notable differences between the two transects despite their proximity and similar history. The wide, flat shape of the QT cline in the transitional forest transect was characteristic of a neutral cline (Barton and Hewitt 1985), suggesting that barriers to gene flow were either absent or very weak in this area. In contrast, the QT cline along the coastal transect showed steepness typical of a cline that was either recently formed, or is maintained by selection (Barton and Hewitt, 1985). We can estimate the dispersal rate from the QT clines using the equation $T=0.35(w/L)^2$ where T is the number of generations necessary to create a cline w metres wide with a rate of L metres of gene flow per generation (Endler 1977). Assuming identical timing of secondary contact in the two transects (see above), the dispersal distance was 4.5 times larger in the transitional forest transect than in the coastal transect. This result strongly suggests restriction of gene flow in the coastal transect.

Endogenous or exogenous selection, or a combination of both, can maintain clines in secondary contact zones long after initial contact (Dasmahapatra et al., 2002; Phillips et al., 2004). Environmental selection has been shown to be important in anoles and can restrict gene flow (Ogden and Thorpe, 2002). Habitat variation (in the shape of a north-south gradient) and QT pattern were correlated on the coastal transect. Compared to the very steep ecotonal transitions in bioclimate and habitat between coast and mountain that drive ecological selection on QTs in A. roquet (Ogden and Thorpe, 2002; Thorpe et al., 2008) the difference in bioclimate and habitat between extreme ends of the coastal transect was relatively small, and the change along the transect was a gentle, linear gradient. Hence, exogenous selection may be a contributing factor, but on its own it is unlikely to explain the sharp QT transition observed here.

Endogenous selection in secondary contact zones is evidenced by coincidence and con-

currence of multiple character clines independent of environmental variation (Brumfield et al., 2001; Phillips et al., 2004), and occasionally by phenotypic anomalies (Brumfield et al., 2001). We found a strong correlation between lineage and QT patterns on the coast, and this finding was also corroborated by the coincidence and concurrence of mtDNA and QT cline centres and widths. Further, at the point where the lineages meet on the coast (locality A05) unusual pigmentation anomalies occur on the head and neck: blotches of white, or skin lacking scales and pigmentation (pers. obs). Anomalies of this type were previously described for this region (and this region only) by Lazell (1972); in secondary contact genetic differences that evolve in allopatry can lead to genic incompatibilities, where alleles tend to function better in the population they are sourced from, but can nevertheless be functional in a different genetic background (Coyne and Orr, 2004). This process can occur with or without natural selection, however when driven by natural selection the process is likely to occur more rapidly (Coyne and Orr, 2004).

On this relatively fine spatial scale, the QTs on the coast were bimodal which could suggest assortative mating (Jiggins and Mallet, 2000). Although it may occur in lizards (Bleay and Sinervo, 2007), it is not readily demonstrated and has yet to be unambiguously shown to occur in anoles (Tokarz, 1995). Nevertheless, several experimental studies of male dewlap colouration and display behaviour (dewlapping and head bobbing) suggest the possibility of female choice (Sigmund, 1983; Crews, 1975; Fleishman, 1992; Tokarz, 1995). Moreover, adaptation to habitat may include changes in dewlap hue to increase visibility/detectability in specific light conditions, which may influence anole interactions (Leal and Fleishman, 2002, 2004) Hence, assortative mating as a contributor to the QT pattern merits further investigation.

Results for the coastal transect thus conformed to the expectations of the allopatric speciation model, where differences built up in allopatry are maintained after secondary contact. Both endogenous and exogenous selection may contribute to maintaining the QT cline, and our results are consistent with a strong barrier to gene flow. In contrast, we did not find evidence consistent with barriers to gene flow on the transitional forest transect. Patterns of lineage and QT variation were weakly correlated, but this was not supported by a more detailed analysis of their clines which had different centres and widths. Hence, the transitional forest transect, like the montane rainforest transects (Ogden and Thorpe, 2002; Thorpe et al., 2008) suggest that the populations do not behave consistently with an allopatric model of speciation. There is overwhelming evidence of the importance of natural selection in shaping population divergence in Lesser Antillean anoles (see references above) and the extent of convergent versus divergent selection along these two transects

may contribute to explain the difference between them. Strong convergent selection in the transitional forest habitat may have eradicated the effects of allopatric divergence, as occurs where these two forms meet in the mountains (Ogden and Thorpe, 2002; Thorpe et al., 2008). Conversely, the absence of strong convergent selection, and the presence of some divergent selection either side of the secondary contact on the coast, may allow the perpetuation of differences built up in allopatry.

CONTRASTING DYNAMICS OF SECONDARY CONTACT ZONES OF *Anolis roquet* IN NORTHEASTERN MARTINIQUE

6.1 ABSTRACT

In a previous study we surveyed patterns of quantitative traits variation and mtDNA lineage distribution on parallel and geographically close transects across a zone of secondary contact between allopatric lineages of the lizard *Anolis roquet* on the island of Martinique. The results suggested allopatric influence on quantitative traits variation on the coast, but not in the transitional forest transect. Here we used the same two transects to study another two sets of characters, dewlap hue and microsatellite variation. Dewlap hue has been proposed as a potential trait for sensory drive, carrying implications for sexual selection and speciation in *Anolis* lizards. Dewlap data was analysed with multivariate statistical techniques. Nine microsatellite markers were used to test gene flow predictions using Bayesian assignment techniques. Sigmoid clines were fitted to both dewlap hue and microsatellite data. The results confirm: i) the absence of barriers to gene flow in the transitional forest, and ii) the presence of barriers to gene flow on the coast, in accordance with an allopatric model of divergence. Moreover the dewlap cline was found to be displaced from all other characters on the coast. A selective advantage scenario is proposed for the observed patterns of dewlap hue on the coast, where detectability of dewlap hue in certain light conditions may allow positive selection to occur. This study conforms that allopatry may play a significant role in *Anolis* lineage diversification where local natural selection pressure permits.

6.2 INTRODUCTION

The fate of divergent geographically isolated populations that attain secondary contact has received considerable theoretical (Endler, 1977; Barton, 1983; Barton and Hewitt, 1985) and empirical attention (Harrison and Rand, 1989; Szymura and Barton, 1986; Dasmahapatra et al., 2002; Brumfield et al., 2001; Babik et al., 2003; Vines et al., 2003; Sequeira et al., 2004; Leache and Cole, 2007) because of the potential insights that may be gained into the process of speciation (Harrison, 1991; Nurnberger et al., 1995). At initial secondary contact, genetic and phenotypic characters frequently form steep clines between the two forms. In the absence of selection against hybrids, or with only weak selection, free movement between populations produces flat and discordant character clines (Endler, 1977; Barton and Hewitt, 1985). On the contrary, a balance between dispersal and strong selection against hybrids can maintain steep clines long after initial secondary contact, and in this case multiple character clines will share centres and width (Barton, 1983; Barton and Hewitt, 1985; Phillips et al., 2004). Moreover, if there is strong selection against hybrids continued divergence is a possibility. Strong selection can effectively impede the spread of selectively neutral or negative alleles, however, positively selected alleles may be able to spread through a hybrid zone (Pialek and Barton, 1997; Brumfield et al., 2001). The spread of positive alleles is often rapid and may therefore not be detected. Nevertheless, some studies of secondary contact zones have shown evidence of positively selected traits (Parsons et al., 1993; Rohwer et al., 2001; Brumfield et al., 2001; Uy and Stein, 2007).

Secondary contact between previously isolated populations may occur during climatic oscillations when populations from different refugia recolonise previously unoccupied habitats (Hewitt, 2001), or when vicariant events connect two previously isolated areas. The island of Martinique in the Lesser Antilles is a good example of the latter. Four peripheral precursor islands (two younger and two ancient) were merged by the uplifting of a central region some 1-1.5 million years ago, forming the present-day island of Martinique (Andreieff et al., 1976; Bouysse, 1983; Maury et al., 1990; Sigurdsson and Carey, 1991; Thorpe et al., 2008). Evidence from the mtDNA structure of a lizard endemic to this island, *Anolis roquet*, complements geological data, and suggests that precursor islands were colonised by lizards that had evolved in allopatry for a significant period of time (6-8 million years) at the time of the merging (Thorpe and Stenson, 2003; Thorpe et al., 2008). There is now a strong *A. roquet* mtDNA structure on the island, with four major mtDNA lineages and three major secondary contact zones (Thorpe and Stenson, 2003).
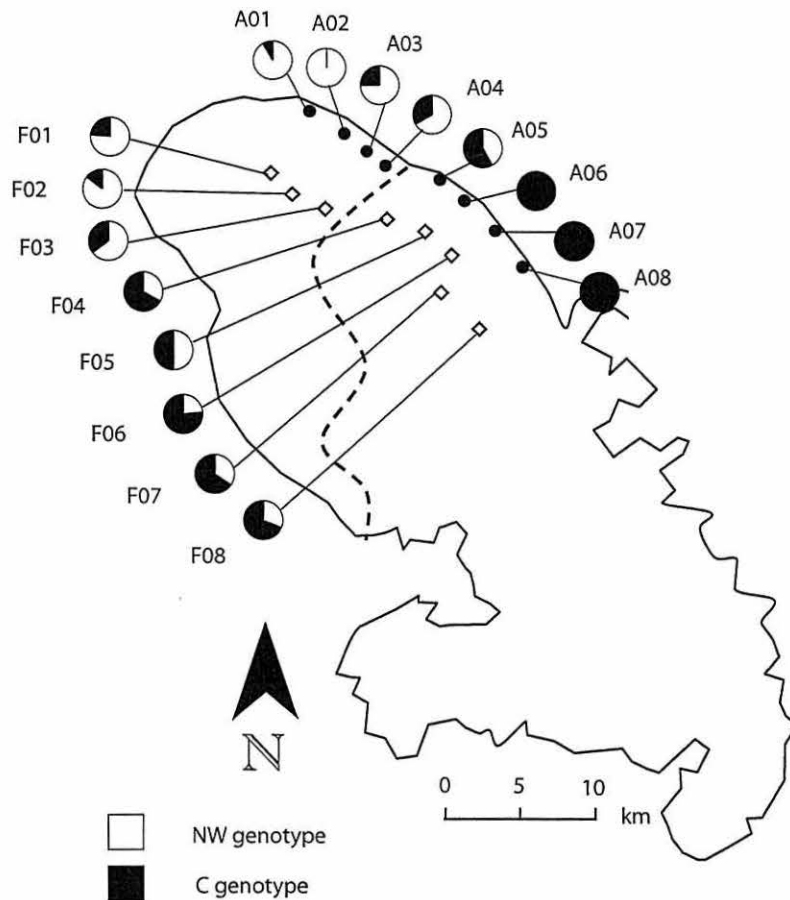
Figure 6.1: Map of Martinique, showing the sampling localities on the two transects. The mitochondrial DNA lineage boundary between the northwestern and central lineage is indicated by the dashed line. Pie charts show the STRUCTURE genotype assignment for two putative populations from the separate analyses of the two transects (see text for details).

The two most distant mtDNA lineages of *A. roquet* (7.9% uncorrected divergence between the central (C) and the northwestern (NW) lineage) come into contact along a geographical boundary that bisects the north of Martinique (Figure 6.1) (Thorpe et al., 2008; Johansson et al., 2008c). Along this boundary there is considerable bioclimatic change; habitat changes from xeric coastal habitats to montane rainforest via transitional forest, within relatively short distances. This demonstrates the typical environmental zonation that is observed on mountainous Lesser Antillean islands (Beard, 1948). The Martinique anole, like the anoles on the other mountainous Lesser Antillean islands, shows marked geographic variation in hue and pattern, as well as scalation, body dimensions and colour (Lazell, 1972; Thorpe and Malhotra, 1996; Thorpe and Stenson, 2003; Thorpe et al., 2004). Overall, the geographic variation in quantitative traits in anoles from Martinique, and other

similar Lesser Antillean islands, is associated with environmental zonation rather than phylogeographic lineage (Malhotra and Thorpe, 2000; Thorpe and Stenson, 2003). This is interpreted as natural selection for current conditions, and recently it has been suggested that natural selection may be the main force driving differentiation in *A. roquet* (Ogden and Thorpe, 2002; Thorpe et al., 2008). However, there are exceptions to these general patterns (Thorpe and Stenson, 2003; Johansson et al., 2008c), and the secondary contact zones between *A. roquet* lineages on Martinique hence provides a natural setting to study how allopatric divergence and natural selection act on different traits.

In northeast Martinique, the contact zone passes through a range of environmental zones. Here, the C and NW mtDNA lineages come into secondary contact. A recent study of mtDNA and quantitative traits (QT) patterns along two parallel transects, (one on the coast and one a few kilometres inland in the transitional forest (Figure 6.1), revealed contrasting patterns of interactions where the two lineages meet in secondary contact (Johansson et al., 2008b). The transects most likely share geological history, due to their proximity and relative positioning (Johansson et al., 2008c). On the coast the near bimodal QT pattern appears to be consistent with an allopatric model of divergence, with selection against morphological hybrids, whereas there was no indication of barriers to gene flow in the transitional forest. Instead, selection appears to be more convergent in the transitional forest, eroding any effects of allopatric divergence, a pattern that is also observed in the montane rainforest (Ogden and Thorpe, 2002; Thorpe et al., 2008). In contrast, natural selection on the coast appears to be relatively more divergent, allowing continued divergence of the lineages (Johansson et al., 2008c).

A trait that may be particularly sensitive to selective forces in some species of *Anolis* lizards is the dewlap hue. Almost all adult male anoles possess a dewlap, which is a specialized thin, flat, often colourful and retractable skin fold that is exclusively used for signalling (Losos and Chu, 1998; Nicholson et al., 2007). Dewlap hue has also been shown to exhibit considerable interspecific, and in some anoles intraspecific, variation in hue (Thorpe, 2002; Thorpe and Stenson, 2003; Nicholson et al., 2007). One interpretation of this variation is that it is a result of sensory drive (Leal and Fleishman, 2004). The sensory drive hypothesis is based on the premise that natural selection favours effective signals, hence signalling traits may readily diverge in order to increase their effectiveness in local habitat. In particular, sexual selection acting on signalling traits is frequently invoked to explain signal diversification and speciation, for example in the adaptive radiation of African cichlids (Maan et al., 2006), and in divergence of sticklebacks (Boughman, 2001). A recent study of montane and xeric coastal areas of Martinique with documented strong

and convergent natural selection pressure suggested that dewlap hue variation, similar to quantitative traits variation, is shaped by strong natural selection for habitat type (Thorpe et al., 2008).

Considering the potential for sexual selection on dewlap hue (for detectability in habitat) we predict that dewlap hue will reflect the stronger convergent natural selection pressure in the transitional forest, and therefore be similar along the transect. However, if there is selection for increased detectability of dewlap hue, relatively less convergent selection pressure may allow for the dewlap hue to either reflect the allopatric past, or for positive selection to shape variation across the zone. To see if the variation in dewlap hue (and in the previously analysed characters) is reflected in patterns of genetic variation we used nine polymorphic microsatellites to test the prediction of gene flow in the transitional forest and barriers to gene flow in accordance with allopatry on the coast.

6.3 METHODS

6.3.1 SAMPLING AND DNA EXTRACTION

Forty-eight lizards were hand caught and tail biopsies collected at 16 localities distributed over two linear transects that traversed the lineage boundary (Figure 6.1). The tail biopsies were stored in 100% ethanol until DNA extraction. A total of 765 DNA samples were extracted from tail tips using either Qiagen DNEasy Blood and Tissue kit (Qiagen, Germany) following the manufacturers instructions, or the Chelex method outlined by Estoup et al. (1996).

6.3.2 MICROSATELLITE ANALYSIS

All DNA samples were genotyped at nine microsatellite loci (primer concentrations: 0.05 µM for ARO-035, ARO-062, ARO-065 and ARO-HJ2, 0.1 µM, for ARO-120 and 0.2 µM for ABO-P4A9, AAE-P2F9 and ALU-MS06) (Ogden and Thorpe, 2002; Gow et al., 2006; Johansson et al., 2008a) in 5 µl multiplex PCR reactions using Qiagen Multiplex PCR kit (Qiagen, U.S.A.). The manufacturers recommendations for multiplex amplification were followed, with the exception of an annealing temperature of 55°C. Amplified PCR products were analysed on an ABI 3130*xl* genetic analyser with the internal size

standard LIZ-600, and the resultant genotypes scored using the software GENEMAPPER v4.0 (Applied Biosystems, U.S.A.).

Microsatellite data were analysed independently for each transect. Exact tests for conformity to Hardy-Weinberg equilibrium (HWE) (Guo and Thompson, 1992) and linkage disequilibrium (Slatkin and Excoffier, 1996) were performed with the software program ARLEQUIN v3.1. (Schneider et al. (2000): http://lgb.unige.ch/arlequin/). A Bonferroni correction was applied to control for Type I errors as a result of multiple testing.

We analysed genetic structure with the software STRUCTURE v2.1 (Pritchard et al. (2000): http://pritch.bsd.uchicago.edu/structure.html). STRUCTURE is a Bayesian clustering software that uses a genetic inheritance model to minimise HWE and linkage within a number of optimal clusters. Hence, underlying population substructure can be detected without a priori definition of the populations. We set number of populations (K) from 1 to N + 1 (where N represented the number of sampled populations), and performed 10 independent runs using the default admixture model with a burn-in of 100,000 followed by 400,000 post burn-in iterations. We used the individual q-values to assign individuals to a) two putative populations b) plot the individual q for two putative source populations, to graphically represent the amount of admixture on each transect. The optimal number of clusters was determined from the posterior probabilities generated by STRUCTURE and confirmed using the method of Evanno et al. (2005), which applies an ad hoc statistic ΔK, that is based on the rate of change between successive K-values.

6.3.3 SPECTROPHOTOMETRICAL ANALYSES OF DEWLAP HUE

We used spectrophotometrical methods to measure *A. roquet* dewlap hue (Macedonia, 2001; Thorpe, 2002; Macedonia et al., 2003; Leal and Fleishman, 2004). A 200 μm receptor fibre was held at a 45°angle to the surface of the dewlap and the diffuse reflectance from the surface was recorded as a percentage of a WS-2 white reflectance standard, using an AvaSpec-2048 spectrometer with an AvaLight-Xe xenon pulsed lightsource (Avantes, Netherlands). For each locality, three recordings from each of the anterior and posterior regions of the dewlap were taken from ten adult male lizards. The recordings produce data on hue and intensity that can be extracted using the matrix-algebraic procedure described in (Thorpe, 2002), a method that aims to extract independent wavelength segments that can be compared across large samples of individuals at numerous localities (Thorpe, 2002; Thorpe and Stenson, 2003). The protocol by Thorpe et al. (2008) was followed. The average of the three readings from each region was used for analyses. Transects were

analysed separately, by inputting the colour segments into a canonical variate analysis (CVA) with locality as grouping variable (Thorpe and Stenson, 2003; Thorpe et al., 2008) using BMDP.

6.3.4   CLINE FITTING

Clines were fitted to the centroids from the CVAs of dewlap hue, and to microsatellite data. The centroids from the CVAs were rescaled between 0-1 (Brumfield et al., 2001; Leache and Cole, 2007), and clines fitted as described below. For the microsatellite data we used the individual assignment probabilities (q-values) produced by STRUCTURE (for $K = 2$) to partition individuals into one of the two putative populations with a probability of 0.5 or more. For each locality the number of individuals belonging to either population were summed up, transformed to proportions and scaled from 0-1 (Babik et al., 2003; Yannic et al., 2008). Clines were fitted to the genotype frequencies (Babik et al., 2003; Yannic et al., 2008) and to the CV centroids (Leache and Cole, 2007) using the software ANALYSE v 1.3 (Barton and Baird (1999):http://www.biology.ed.ac.uk/research/ institutes/evolution/software/Mac/Analyse/Version1.3.html.). Allowing centre and width to vary, we used fixed values for P_{min} and P_{max} (where P_{min} and P_{max} denote minimum and maximum character frequencies in the tail end of a cline) and searched for the best fit over 2000 iterations, as indicated by the generated log likelihood value. The support values option in ANALYSE 1.3 was used to generate support limits within 2 likelihood units, which is equivalent to 95% confidence limits. Significant coincidence and concordance between different clines were determined if centre and width values from one cline could be found within 95% confidence limits (two units of likelihood) of the other cline and vice versa (Dasmahapatra et al., 2002).

6.4   RESULTS

6.4.1   COASTAL TRANSECT

There were no consistent and significant departures from linkage or HW equilibrium in the sample. The best likelihood value from the STUCTURE analysis was obtained for $K = 2$, similarly, Evanno et al. 's (2005) ad-hoc test for the correct number of subpopulations supported $K \leq 2$. The genotype partitioning for $K = 2$ shows that most individuals in localities A01-A03 were classified into one cluster with a probability of 0.5 or more, and
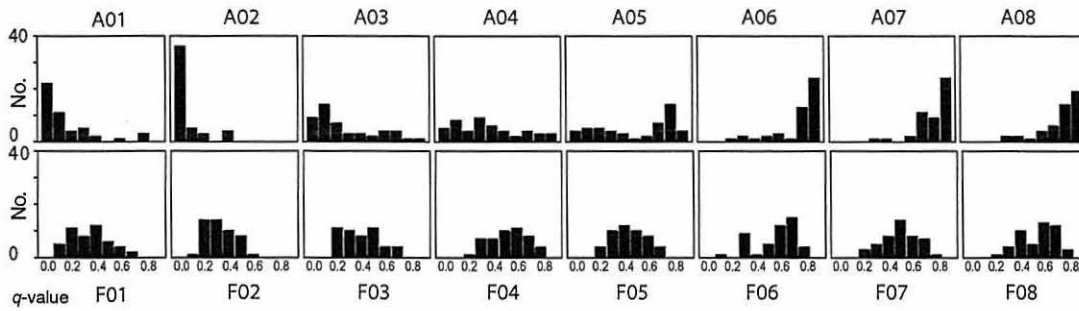
Figure 6.2: q-values from the STRUCTURE analysis, for $K = 2$. The top row represents the coastal transect and the bottom row represent the transitional forest transect. The Bayesian assignment shows that there are predominantly NW or C genotypes ($q < 0.1$ (NW) or $q > 0.9$ (C) in the localities at each end of the coastal transect and mixed ancestry in the centre. In contrast, there are no parental genotypes at any locality in the transitional forest transect.

that all individuals in localities A06-A08 classified into a second cluster. Locality A04 and A05 represent the changeover between assignment of individuals predominantly into either of the two populations (Figure 6.1). The q-values from the STRUCTURE analysis revealed that there is a high proportion of mixed genotypes across the transect, particularly in the central localities (A03-A05), however the high occurrence of parental genotypes ($q < 0.1$ and $q > 0.9$, Pritchard et al. (2000)) at either end of the transect suggests the existence of distinct NW and Central genotypes (Figure 6.2). The corresponding cline of the microsatellite data takes on a sigmoid shape with a width of 6704 m and a centre at 6767 m from the transect start (Figure 6.3a, Table 6.1).

Dewlap hue canonical variate scores show a cline across the transect, where there is considerable total change across the transect, amounting to 4.2 pooled within-group standard deviations (Figure 6.4a). There is overlap of scores along the transect. Qualitatively, anterior dewlaps at the southern extreme of the transect reflect longer wavelengths, whereas anterior dewlaps in the northern extreme of the transect reflect more medium and short wavelengths (Figures 6.5a and b, Table S1- Appendix C). The cline in dewlap hue takes on a sigmoid shape with a width of 6489 m and a centre at 5333 m from the transect start (Figure 6.3a, Table 6.1).

The centres of the dewlap hue and nDNA clines were close, but did not coincide, the dewlap hue being centred 1.4 km closer to the transect start than the nDNA cline. The widths between the two clines concur, the microsatellite cline being approximately 200 m wider than the dewlap hue.

a) Coastal transect

b) Transitional forest transect
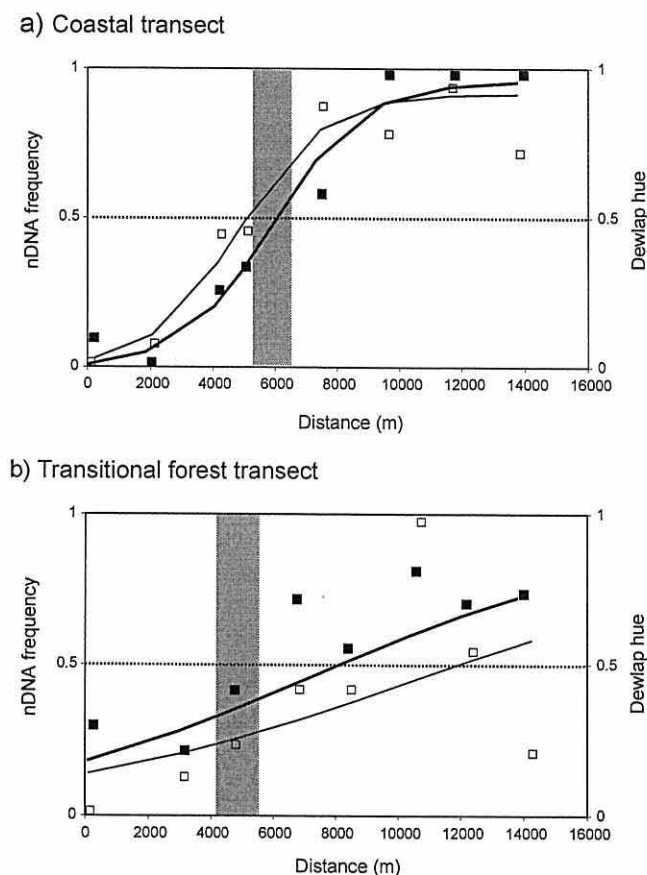
Figure 6.3: Nuclear DNA and dewlap colour clines for both transects. Closed squares and thick line = nDNA, open squares and thin line = dewlap. Cline centres (frequency = 0.5) can be found where the clines intersect the central dotted line. a) Coastal transect: both clines are sigmoid and widths concur, but centres do not coincide. The nDNA cline centre coincides with the geological boundary (indicated by the grey shading), whereas the dewlap cline is displaced approximately 1.4 km north of the nDNA centre. b) Transitional forest transect: both clines are flat, and do not share centres or widths. Neither cline centre coincide with the geological boundary (indicated by the grey shading).

6.4.2 TRANSITIONAL FOREST TRANSECT

There were no consistent and significant departures from linkage or HW equilibrium in the sample. The STRUCTURE genotype assignment (probability >0.5) for $K = 2$ suggests that there is very little substructure in this sample (Figure 6.1). The q-values revealed that there were no genotypes that were classified as parental genotypes, hence all individuals had mixed ancestry (Figure 6.2). The best likelihood value was obtained for $K = 1$, similarly Evanno et al. 's (2005) ad-hoc test for the correct number of subpopulations supported $K \leq 2$. The corresponding nDNA cline was flat and wide, with a width of 23835

Table 6.1: Cline widths and centre positions (metres from transect starts) are given for both transects, with best log likelihood for the estimations. Support limits are in parenthesis.

	Width (m)	Centre (m)	log likelihood
Coastal			
nDNA	6704 (5659-8174)	6767 (6227-7314)	-13.47
Dewlap hue	6489 (5669-7044)	5333 (5221-5448)	-2.01
Transitional forest			
nDNA	23835 (18231-31564)	8030 (6744-9363-6052)	-10.57
Dewlap hue	28137 (26749-29605)	11745 (11469-12031)	-20.02

m, and a centre at 8030 m from the start of the transect (Figure 6.3b, Table 6.1).

There was no strong pattern in the dewlap hue data in the transitional forest transect (Figure 6.4b). Locality scores overlap extensively and the change between extreme ends of the transect amounted to 1.0 pooled within-group standard deviations. A high CV score indicated more green and blue transmittance from the posterior dewlap and more UV1 from the anterior dewlap, and less transmittance of blue and UV (Figures 6.5c and d, Table S2 - Appendix C). The cline from this data was wide and flat with a width of 28137 and a centre 11745 m from the start of the transect (Figure 6.3b, Table 6.1). Microsatellite and dewlap hue clines did not coincide, the microsatellite cline centre being located 2319 m to the north. Widths did not concur, the dewlap hue being 6927 m wider (Figure 6.3b, Table

a) Coastal transect

b) Transitional forest transect
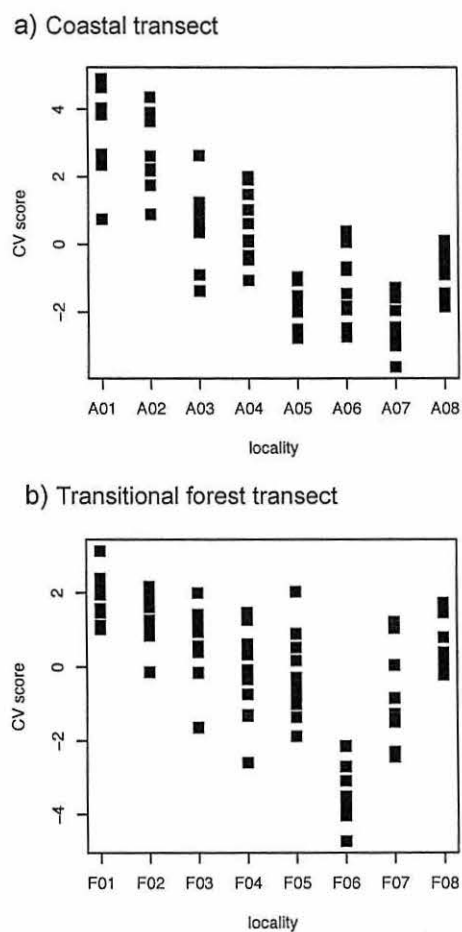
Figure 6.4: Canonical variate scores for the dewlap hue for both transects. CV units are within-group standard deviation a) Coastal transect: there is a clinal pattern in the data. b) Transitional forest transect: no directional pattern is discernible, and scores overlap along the transect.
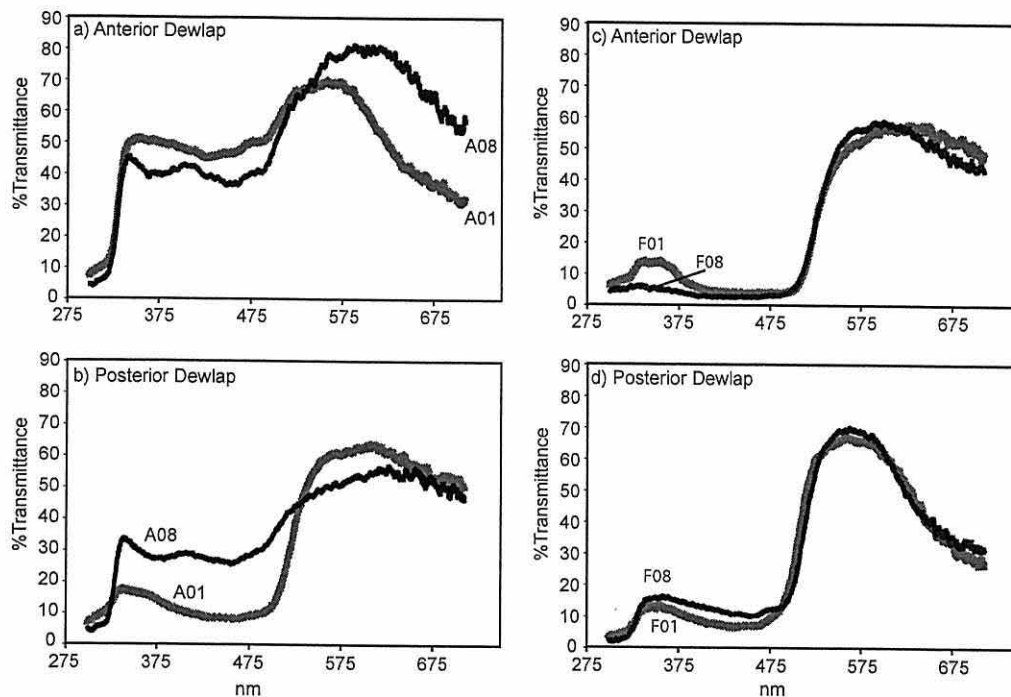
Figure 6.5: Dewlap hue traces. a) Coast: the trace from typical anterior dewlap from locality A01 is higher in UV, blue and green, compared to the trace of the typical dewlap from locality A08 which peak in the yellow/orange and red parts of the spectra. b) Coast: the same pattern is present in the traces from typical posterior dewlaps from these localities. c) Transitional forest: the traces from typical anterior dewlaps are similar at each end of the transect, with slightly more UV at locality F01. d) Transitional forest: the typical traces from posterior dewlaps are indistinguishable between the two extreme localities (F01 and F08).

6.5 DISCUSSION

When geographically isolated populations come into secondary contact, they can either already be reproductively isolated, continue to evolve reproductive isolation, form stable hybrid zones or simply fuse (Harrison, 1991). In this study, the transitional forest transect appears to be an example of a fusing hybrid zone; we found that neither genetic data nor dewlap hue demonstrated evidence of past allopatry. In addition to the nDNA and dewlap hue, clines have previously been fitted to mtDNA and quantitative traits (Johansson et al., 2008c). Of these four characters only one (mtDNA) forms a sigmoid cline, and none of the cline centres coincide nor do their widths concur (Johansson et al., 2008c). The persistence of the mtDNA cline may be attributed partly to female philopatry (Johansson et al., 2008b), and partly to the small effective population size of the marker that allows for a stronger geographical structure to be maintained (Funk and Omland, 2003). The

results here are hence consistent with a previous study by Johansson et al. (2008c) of the same transect, that suggested that convergent natural selection has eradicated the effects of allopatric divergence, leading effectively to neutral diffusion of the genes between the previously isolated populations. This also occurs where these lineages meet in the rainforest, and contributes to the evidence for the important role of natural selection in shaping geographical variation and population divergence in Lesser Antillean anoles (Ogden and Thorpe, 2002; Thorpe et al., 2008).

In contrast, genetic data support the existence of partial reproductive barriers on the coast, indicating that this may be a stable hybrid zone, or one where reproductive isolation may have an opportunity to evolve (Harrison, 1991). The assignment analysis shows that there are distinct NW and C nDNA parental genotypes at either end of the transect with extensive hybridisation in the central localities. Furthermore, the nDNA cline centre coincided with the geological boundary, and therefore with the mtDNA and quantitative trait clines (mtDNA centre: 6715, 95% confidence 6344-7155, QT centre 6450, 95% confidence 6193-6767), however the nDNA cline was wider (mtDNA width: 3016, 95% confidence limit 2549-3587, QT width 2990, 95% confidence 2057-4044) (Johansson et al., 2008c). Coincidence of multiple character clines suggest selection against hybrids, and this part of the contact zone fits the description of a tension zone that is maintained by a balance of selection and dispersal (Barton and Hewitt, 1985). Furthermore, the patterns are consistent with an allopatric model of divergence; three of the clines coincide with a geological boundary (Johansson et al., 2008c).

Different secondary contact dynamics in different areas of the same zone of secondary contact have been observed in other organisms, e.g. the *Bombina bombina/ B. variegata* hybrid zone in Romania is mosaic (Vines et al., 2003), whereas in Poland, Croatia and Ukraine there are narrow clines with extensive pure populations on either side of the zone of contact (Szymura and Barton, 1986, 1991; MacCallum et al., 1998). In this study, the difference between patterns is very pronounced considering the short distances that separate the two transects. Natural selection for habitat is most likely responsible for the contrasting patterns and dynamics between the transects. The contact zone studied here travels through montane rainforest, to the coastal habitat, via transitional forest. On the coast the habitat differ either side of the contact zone, in the transitional forest there is comparatively less difference (Johansson et al., 2008c), and where the lineages meet in the rainforest the habitat is very similar (Ogden and Thorpe, 2002; Thorpe et al., 2008).

On the coastal transect, the dewlap hue cline takes on a sigmoid shape, suggesting that this cline, similar to nDNA (see above), mtDNA and QT clines (Johansson et al., 2008c),

is maintained by selection. Furthermore, the CVA shows that there is considerable difference in hue between the north and south of the transect. The width and shape of the cline is similar to that of nDNA, however, the centre of the dewlap hue cline is displaced a significant distance north of the other cline centres. Non-coincident clines have been reported in a number of hybrid zones, and are classified into two categories by their spatial pattern. The first category, staggered clines, is typified by a series of individual character clines that are spaced approximately one cline width apart from each other (Jaarola et al., 1997). This type of pattern has mainly been observed between mtDNA and/or chromosomes in shrews or mice, and has been attributed to zonal raciation and heterozygote disadvantage (Searle, 1991; Searle et al., 1993; Harrison, 1991). In the second category, clines are non-coincident due to asymmetrical introgression. In this case the cline centre of one or a few characters is displaced far outside a main cluster of clines, as occurs in this study. Hybrid zone movement, due to environmental change (Dasmahapatra et al., 2002) or into areas of low population densities (Barton and Hewitt, 1985), neutral introgression and founder events (Butlin and Neems, 1994), genetic dominance of a trait (Brumfield et al., 2001; Rohwer et al., 2001) or selective advantage (Brumfield et al., 2001; Rohwer et al., 2001) have been invoked to explain observed patterns of discordant clines due to asymmetrical introgression.

Of these possibilities, it seems unlikely that this hybrid zone has moved substantially, considering the close association between three of the four clines and a particular geological boundary (mtDNA) and the same geological boundary are also associated in the transitional forest transect (Johansson et al., 2008c), and in the montane rainforest (Ogden and Thorpe, 2002; Thorpe et al., 2008). However, hybrid zone movement can never be fully excluded if long term historical data of distribution is absent, as it is here. An other possibility is selective advantage (Brumfield et al., 2001; Rohwer et al., 2001) which may be speculated from the use of the dewlap as a signalling organ. The effectiveness of a dewlap signal appears to be dependent on the light conditions of the habitat; for example, a recent study of *A. cristatellus* found an association between dewlap intensity and in specific habitat conditions, leading the authors to suggest a role for sensory drive in *Anolis lizards* (Leal and Fleishman, 2004). Moreover, a behavioural study examined the impact of contrast between the background and the dewlap on inter-sexual responses in *A. carolinensis* (Sigmund, 1983). This study suggested that where the dewlap contrasts sharply against the background (e.g. red dewlap against a green background) a stronger response is elicited (Sigmund, 1983). If a specific hue of the *A. roquet* dewlaps does indeed confer selective advantage, it would normally be expected to spread throughout the hybrid zone and progress rapidly to fixation. However, the hue may not have selective advan-

tage in all habitats along the transect; the existence of a bioclimatic gradient and habitat change from southern xeric to northern mesic littoral woodland along the transect (Johansson et al., 2008c) may render the trait of neutral value, or disadvantage, past certain habitat conditions, and therefore impede the spread of the trait. Although this prospect is speculative, it has been observed to occur with secondary sexual plumage traits in the *Manacus vitilinus/M. candei* hybrid zone (Stein and Uy, 2006a,b; Uy and Stein, 2007). Other possibilities are neutral drift (Butlin and Neems, 1994), including founder effects (Gyllensten and Wilson, 1987; Barton, 1983), which cannot be excluded considering the relatively small shift of the cline centre, and finally, dominance of one or more of the southern dewlap hue traits. This could cause the observed pattern, even if the underlying allele frequencies are coincident with other clines (Brumfield et al., 2001). However this hypothesis cannot be tested without crossing trials.

This study shows contrasting dynamics between closely related populations of *A. roquet* coming into secondary contact in different habitats. In the transitional forest the nuclear genetic and dewlap hue data confirmed that there is free movement of genes between populations. In contrast, genetic data suggested that partial reproductive barriers exist on the coast. The difference between the transect is most likely contingent on stronger convergent selection in the transitional forest, and on less convergent selection and possibly divergent selection on the coast (Johansson et al., 2008c). These results strongly suggest that allopatric divergence may play a significant role in divergence and speciation of Lesser Antillean anoles if natural selection allows.

GENERAL DISCUSSION

7.1 BACKGROUND AND RATIONALE

The relative roles of geographical mode and isolating barriers in speciation have been, and remain, the subject of contentious debate in evolutionary scientific literature (Coyne and Orr, 2004). Studies on natural systems are important contributors to this debate. The *Anolis roquet* lineage complex on Martinique provides a good system for the study of the effects of natural selection and allopatry on population divergence. There is strong geological and molecular evidence for long periods of allopatric divergence between lineages on precursor islands, and subsequent secondary contact between the lineages (Thorpe and Stenson, 2003; Thorpe et al., 2008). There is also strong evidence for the effect of natural selection in shaping geographic variation of morphology (Ogden and Thorpe, 2002). The study of secondary contact dynamics can provide insight into past and current processes that drive differentiation (Alexandrino et al., 2005).

7.2 DEVELOPMENT AND USE OF MOLECULAR MARKERS

The development of cost-effective methods for the isolation and screening of species-specific molecular markers, and improvements in computing technology, have made molecular markers almost ubiquitous in the field of population genetics (Selkoe and Toonen, 2006). For this thesis, two molecular markers were used; diagnostic PCR-RFLPs and hypervariable microsatelllite markers. The application of a diagnostic PCR-RFLP assay provided a reliable and rapid method for individual assignment to mtDNA clades, a method that is commonly used in hybrid zone studies (Ruegg, 2007). Twenty-two microsatellite markers were isolated *de novo* from seven species in the *roquet*-series of anoles, using a standard enrichment protocol (Gardner et al., 2001). Nine microsatellites from this panel and the microsatellites developed by Ogden et al. (2002) were used for population genetic screening. In spite of some known limitations, such as null alleles and the unknown mutation model (Schlotterer, 2000), microsatellites remain the marker of choice in fine-scale population genetic surveys (Selkoe and Toonen, 2006).

7.3 MALE-BIASED DISPERSAL

The decision to test for male-biased dispersal using microsatellite data was informed by the observed discrepancy of mtDNA and nDNA pattern across secondary contact zones

in *A. roquet* (Ogden and Thorpe, 2002; Thorpe et al., 2008). The different properties of the molecular markers, specifically the smaller effective population size of mtDNA, could explain some of the discrepancy. However, sex-biased dispersal is known to be another potential cause of this type of pattern (Castella et al., 2001). Moreover, mtDNA pattern appeared to be remarkably preserved considering the estimated period of 1-1.5 million years of secondary contact (Thorpe et al., 2008). Indeed, microsatellite data showed strong evidence for male-biased dispersal. These results were also consistent with a dispersal study on *A. oculatus* which incorporated a test on sex-biased dispersal (Stenson et al., 2002), and the results are consistent with observations of behaviour in sexually dimorphic species of anoles. This study represents one of the growing number of sex-bias studies in reptiles which provide independent (from mammals and birds) tests of the hypotheses concerning the evolution of sex-biased dispersal (Doughty and Sinervo, 1994a,b; Olsson et al., 1996; Lena et al., 1998; Gardner et al., 2001; Stow et al., 2001; Stenson et al., 2002; Massot et al., 2003; Olsson and Shine, 2003; Chapple and Keogh, 2005; Dubey et al., 2008; Ujvari et al., 2008).

The benefit of male-biased dispersal, when females are philopatric, is at the most basic level inbreeding avoidance (Pusey, 1987), though often influenced by competition (Greenwood, 1980; Dobson, 1982; Perrin and Mazalov, 2000). However, dispersal, including male-biased dispersal, may also have implications for population divergence. With high rates of dispersal, population differentiation is anticipated to progress slower, due to a constant flux of alleles between populations. Selection against dispersing individuals, leading to fewer or no matings, may on the other hand balance the effects of dispersal, allowing the progression of population divergence (Clobert et al., 2001). In organisms with male-biased dispersal and strong female philopatry, female choice (sexual selection) may be a potent selective force for the success of dispersers. Positive selection allows dispersing males the opportunity to mate, whereas negative selection may effectively claim the ultimate cost of dispersal for males, i.e. no matings.

7.4 SECONDARY CONTACT DYNAMICS

There is a large body of evidence for the role of natural selection in shaping geographical variation in Lesser Antillean anoles (Thorpe et al. (2004); Thorpe (2005), and references therein). Indeed, natural selection pressure is so strong that adaptation to habitat was suggested as the main force driving population divergence in *A. roquet* (Ogden and Thorpe, 2002; Thorpe et al., 2008). However, a close examination of a region identified by Thorpe

and Stenson (2003) as a potential exception to this pattern revealed allopatric divergence in patterns of QT, dewlap hue and nDNA variation that persist after secondary contact of formerly isolated lineages. A few kilometres inland, in transitional forest habitat, secondary contact interactions between the same two lineages showed no evidence of past allopatry. The results from the transitional forest transect reflected natural selection, in accordance with results from Ogden and Thorpe (2002).

Two separate issues are pertinent for these results: the different consequences of the interactions between lineages in secondary contact on each transect, and the effect of natural selection pressure between the two transects.

7.4.1 SECONDARY CONTACT INTERACTIONS ON THE COAST AND IN THE TRANSITIONAL FOREST

Hybrid zones have been termed 'laboratories of evolution', and they continue to receive significant research attention (Leache and Cole, 2007; Ruegg, 2007; Yannic et al., 2008; Mullen and Hoekstra, 2008). Hybrid zone studies have recently been carried out in groups with uncertain taxonomies (with regards to species status), such as the the *Ensatina* ring-species (Wake, 1997; Alexandrino et al., 2005), the *Sceloporus undulatus* (Leache and Cole, 2007) the *Sceloporus grammicus* (Sites et al., 1996; Marshall and Sites, 2001) and *Carlia rubrigularis* (Phillips et al., 2004). Such studies can clarify species status, and new classifications may be proposed, as in the case of *Carlia rubrigularis* (Phillips et al., 2004). In some instances, such as in the *Ensatina* complex (Alexandrino et al., 2005), and also in the *Bombina bombina/ B. variegata* hybrid zone, there are different dynamics in different parts of the same secondary contact zone (Szymura and Barton, 1986, 1991; ?)

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In this study, secondary contact lineage interactions were very different on the two transects. On the coast there was evidence of partial reproductive barriers in accordance with an allopatric past. Mitochondrial DNA, QT and nDNA had steep clines with cline centres at the geological boundary, with the fourth character, dewlap hue, having a steep cline with a slightly displaced centre. There were clear groups distinguishable in from the CV analyses of QT variation and dewlap hue. Individuals in localities A05-A08 had similar scores and formed a cohesive group for both characters. This group was different from localities A01-A04, in which individual scores were more variable, and hence there was less cohesion among groups, particularly in the dewlap hue. These patterns suggest the effect of divergent selection for environment (past, present or both), that are maintained

by selection against hybrids after secondary contact. This is a common pattern in hybrid zones (Phillips et al., 2004; Alexandrino et al., 2005), and with strong selection acting on numerous characters hybrid zones are often considered to be stable (Szymura and Barton, 1986, 1991)

The displaced centre of the dewlap hue cline indicated asymmetric introgression, which was interpreted as selective advantage. There has been many studies carried out on the function of dewlap colour in *Anolis* lizards, the most recent suggesting that habitat detectability/visibility drives the evolution of dewlap hue variation in some species (Leal and Fleishman, 2004). The results here suggest that this is a plausible theory in *A. roquet* in some areas of Martinique. Dewlaps transmitting longer wavelengths may be more detectable in some, but not all, of the habitats on the coast, leading to positive selection for this trait. Asymmetric introgression, due to selective advantage, was studied in detail in a hybrid zone between *Manacus vitellinus* and *M. candei* and recently, frequency dependent sexual selection for plumage traits that increase visibility has been observed in these birds (Parsons et al., 1991; Brumfield et al., 2001; Stein and Uy, 2006b,a; Uy and Stein, 2007). Sexual selection may, in cases of selective advantage, weaken the barriers against gene flow in one direction in the hybrid zone. Considering that males are the main dispersers in *A. roquet*, and that they have larger and more colourful dewlaps than females, selection for, and against, males based on dewlap hue may play a significant role in causing the patterns seen on the coast.

The different widths of the character clines on the coast implied that selection acts differently on the different traits. The strongest selection acts on QT, followed by dewlap hue and this selection was evident in the nDNA. Nevertheless, the large number of hybrids in the centre of the transect suggest that there is considerable gene flow along the transect. Different selection on characters are common in secondary contact zones and traits may be maintained as very steep clines in the face of considerable gene flow (Mullen and Hoekstra, 2008).

It was impossible to determine the exact nature of the selection that maintains this hybrid zone. This is a perennial problem in hybrid zone studies, because endogenous and exogenous selection give rise to similar clines (Kruuk et al., 1999). Similar to many surveyed hybrid zones (Szymura and Barton, 1986, 1991; Brumfield et al., 2001; Marshall and Sites, 2001; Alexandrino et al., 2005), it is most likely a combination of selection pressures that maintain the hybrid zone. However, there is a relatively small bioclimatic change between the ends of the transect, compared to other transects surveyed on Martinique (Thorpe et al., 2008). There is also evidence of endogenous selection, and the

pattern of QT variation suggest that there may be assortative mating (Jiggins and Mallet, 2000). It therefore seems likely that a combination of hybrid inferiority and assortative mating may be the main factors that maintain this hybrid zone. Some studies suggest that assortative mating occurs in some species of lizard (Bleay and Sinervo, 2007), however the evidence for mate choice and assortative mating in *Anolis* lizards is equivocal (Tokarz, 1995).

In contrast to the steep clines on the coast, all clines were flat and wide on the transitional forest transect, with the exception of the mtDNA cline. Considering that there is male-biased dispersal in *A. roquet*, mtDNA pattern was expected to be more conserved than the other markers used in this study. The CVAs for QT and dewlap hue variations showed relatively little difference between extreme ends of the transects. The patterns suggest that there is no selection against hybrids, instead strong convergent selection on the lineages (perhaps past, most likely present, or both) has led to homogenisation of the general appearance. Furthermore, low female mate discrimination due to the more similar appearance of lizards in the transitional forest may contribute to the relatively rapid fusion of populations here. A number of studies on different organisms have shown similar effects of convergent selection (Schluter, 2001; Rundle and Nosil, 2005), a process that may lead to ecological speciation in some conditions.

The contrasting secondary contact dynamics on these two transects may carry opposing implications for divergence in *A. roquet*. In the transitional forest the absence of strong selection against hybrids of the two previously isolated lineages means that the lineages appear to be fusing into one panmictic population. On the coast, the strength of selection against hybrids will ultimately determine the persistence of this hybrid zone.

7.4.2 DIFFERENCE IN NATURAL SELECTION PRESSURE BETWEEN THE TWO TRANSECTS

The role of natural and (some types of) sexual selection in population divergence, regardless of biogeographic model, has received a lot of attention in the past decade, leading to a formalisation of the concept of ecological speciation (Schluter, 2001; Rundle and Nosil, 2005). Ecological speciation theory predicts the independent evolution of convergent ecomorphs in similar environments (Schluter, 2001). For example, in Greater Antillean *Anolis* lizards similar ecomorphs (similarity in morphology, ecology and behaviour) have evolved independently on each island, in accordance with structural habitat (Losos and Chu, 1998). On the other hand, divergent selection is also associated with

speciose adaptive radiations, such as the African cichlids (Allender et al., 2003). Here, rapid diversification in signalling traits to increase visibility in specific habitat is though to account for much of the diversity (Allender et al., 2003).

On Martinique, it has been demonstrated that strong natural selection pressure between coast and mountains drive divergence in QT, nDNA (microsatellite markers) and dewlap hue in *A. roquet* (Ogden and Thorpe, 2002; Thorpe et al., 2008), with these characters correlating strongly to patterns of environmental variation (Thorpe et al., 2008). Moreover, convergent selection in extreme habitats has been demonstrated in *A. roquet*, where lizards in xeric coastal habitat are pale with stripes, and lizards in the rainforest are green with white spots, regardless of phylogenetic lineage (Thorpe and Stenson, 2003). Morphological changes in Lesser Antillean anoles have been shown to be genetically controlled and not due to phenotypic plasticity (Thorpe et al., 2005).

In this study, the difference in secondary contact interactions between the two transects appear to governed by the localised natural selection regime. Strong convergent selection in the transitional forest appears to erode the effect of allopatric divergence in *A. roquet*, however weaker convergent selection on the coast may allow the perpetuation of differences built up in allopatry. Considering the evidence for convergent selection on Martinique, it is not unexpected that lizards in the transitional forest are similar in appearance. More surprising was the finding of divergent phenotypes in agreement with allopatric divergence on the coast. This is the sole known region of Martinique where this occurs.

However, there is increasing recognition that local habitat and lineage history may play significant roles for diversification even in organisms where natural selection is accepted as the main cause of differentiation (Lagerhans et al., 2006). For example, Lagerhans et al. (2006) attributes common selective pressures within similar habitat as the main cause of the morphological patterns seen in the Greater Antillean anole communities. However, island/phylogenetic histories and island specific responses to similar environments were also found to represent significant aspects of diversity. Divergent evolution can be observed in closely related taxa that inhabit identical selective environments, due to different colonisation and dispersal histories (Price et al., 2000; Lagerhans et al., 2006; Fukami et al., 2007; Seehausen, 2007).

Moreover, firm geological evidence of secondary contact can be difficult to establish, and lineage history and taxonomic relationships may be indeterminate, especially in rapidly diversifying genera. There have been studies of change in mtDNA and morphology along linear transects in *A. marmoratus* on Guadeloupe (Malhotra and Thorpe, 1994; Schneider,

1996) and island-wide nDNA studies on *A. oculatus* on Dominica (Stenson et al., 2002) which suggest that there may be other instances where allopatric effects may be the cause of observed differences in morphology and restriction of gene flow in Lesser Antillean anoles. However, the geological records for these islands are unreliable due to considerable explosive volcanism, therefore convincing support for secondary contact is absent (Malhotra and Thorpe, 1994; Schneider, 1996; Stenson et al., 2002).

The anoles of the Greater Antilles provide another case where past allopatry may play a part in diversification: a phylogeographic mtDNA and nDNA study of the Cuban green anoles *A. allisoni* and *A. porcatus* have shown evidence of secondary contact. These two closely related anoles are thought to have colonised Cuba at a time when the island was partially submerged. Secondary contact between the species is thought to have occurred in the late Miocene or Pleistocene (Glor et al., 2004). Moreover, hybridisation is known to occur from both genetic and morphological evidence. Such parallels strongly suggest that the causative forces of divergence in some Lesser Antillean and Greater Antillean anoles may be relatively contingent on geographical isolation.

7.5   FUTURE DIRECTIONS

There are several outstanding questions about dispersal in *A. roquet*. Specifically, dispersal distances, total proportion of dispersing individuals from a population and total proportion of male to female dispersers require attention in order to investigate the effects of male-biased dispersal on population divergence in *A. roquet*. Moreover, male-biased dispersal occurs in all lineages and habitats, but that is not to say that rates do not differ between lineages and habitats. A combination of molecular data and mark-recapture studies would ideally be employed to investigate these questions.

Studies on sex-biased dispersal on several species of anole would be interesting in light of the relationship between polygyny and sex-biased dispersal (Greenwood, 1980). A correlation between female density and sexual size dimorphism has been shown in *Anolis* lizards (Stamps et al., 1997); similarly a correlation between female density and male-biased dispersal may be expected if competition drives evolution of sex-biased dispersal in this genus (Dobson, 1982; Perrin and Mazalov, 2000).

Several lines of inquiry specific to to the northeastern region arise from this work. The cause of the very sharp change in QT on the coast, and the pigmentation anomalies, may be clarified by population screening of genes responsible for colour and black markings,

possibly obtainable with the aid the sequenced *Anolis carolinensis* genome.

To understand the different dynamics of the two transects, the change in quantitative traits, dewlap hue and nDNA between coast and mountain but within each lineage, and the corresponding bioclimatic changes may offer insights into selection regimes. Similarly, quantifying the change in mtDNA, QT, dewlap hue and nDNA from the surveyed C/NW boundary in the north to the C/S boundary in the south may shed light on the natural selection regime along the coast.

Endogenous selection against hybrids may be experimentally tested with common garden experiments. With endogenous selection lower survival of 'hybrids' may be expected, compared to 'pure types' when raised at similar condidtions. Similarly, translocation experiments may give insight into exogenous selection regimes along the transects.

A study of the light conditions on the coast and a comparison with dewlap hue similar to the study by Leal and Fleishman (2004) may prove or disprove the theory of selective advantage, together with crossing trails to exclude the possibility of dominance.

Analyses of the other secondary contact zones on Martinique is underway, which will further clarify the roles of allopatry and natural selection in divergence and speciation in this species. The next step from these studies should involve a genomic approach to identifying which genes are involved in speciation and divergence, and how they vary across the island. Moreover, this approach could be extended to a comparative study of genes involved in anole adaptation and divergence on other Lesser Antillean islands.

Furthermore, potential assortative mating and sexual selection in this species require attention. Female choice in *Anolis* lizards has been speculated from various studies, however there has been no unequivocal evidence (Tokarz (1995) and references therein). Recently, it has been shown that there is most likely male preference for novel females in two species of anole, *Anolis sagrei* (Tokarz, 2008) and *Anolis carolinensis* (Orrell and Jenssen, 2002). However, sperm storage and multiple paternity have also been shown to occur in *Anolis sagrei* (Calsbeek et al., 2007), and cryptic female choice through sperm selection has been proposed for this species (Calsbeek and Bonneaud, 2008). Hence, how *Anolis* lizards choose their mates, and the evolutionary implications thereof, would be a suitable area of study for the future.

APPENDIX A

SUPPLEMENTARY TABLES FOR CHAPTER 4

Table S1. Observed (H_O) and Expected heterozygosities (H_E) are given for each locus and population together with the *P*-value of the significance test for Hardy Weinberg. An asterisk indicates a significant departure from Hardy-Weinberg equilibrium at $P < 0.05$ before Bonferroni correction. None are significant after Bonferroni correction.

Populations		AAE-P2F9	ABO-P4A9	AEX-P1H11	ALU-MS06	ARO-035	ARO-062	ARO-065	ARO-120	ARO-HJ2
					Locus					
CA	H_O	0.750	0.393	0.857	0.964	0.821	0.679	0.857	0.821	0.704
	H_E	0.798	0.406	0.908	0.872	0.704	0.712	0.839	0.670	0.822
	P	0.501	0.470	0.480	0.141	0.559	0.395	0.313	0.608	0.005*
CG	H_O	0.816	0.763	0.921	0.838	0.526	0.526	0.711	0.784	0.816
	H_E	0.854	0.720	0.926	0.902	0.554	0.582	0.753	0.767	0.757
	P	0.619	0.777	0.331	0.256	0.208	0.337	0.711	0.743	0.469
Dg	H_O	0.743	0.600	0.943	0.886	0.743	0.824	0.829	0.914	0.743
	H_E	0.815	0.642	0.952	0.940	0.718	0.718	0.861	0.817	0.764
	P	0.398	0.372	0.885	0.209	0.437	0.465	0.299	0.894	0.343
Ea	H_O	0.896	0.292	0.896	0.833	0.458	0.708	0.854	0.688	0.771
	H_E	0.864	0.345	0.907	0.864	0.506	0.740	0.859	0.787	0.826
	P	0.604	0.034	0.914	0.282	0.089	0.874	0.077	0.189	0.023*
Gg	H_O	0.875	0.719	0.938	0.906	0.813	0.656	0.719	0.656	0.688
	H_E	0.884	0.760	0.909	0.901	0.669	0.604	0.797	0.668	0.823
	P	0.453	0.357	0.935	0.828	0.847	0.910	0.653	0.858	0.054
Ia	H_O	0.829	0.707	0.927	0.927	0.683	0.317	0.829	0.732	0.780
	H_E	0.856	0.737	0.909	0.918	0.708	0.413	0.832	0.711	0.816
	P	0.985	0.233	0.887	0.197	0.595	0.002*	0.810	0.393	0.803
Je	H_O	0.860	0.628	0.860	0.907	0.698	0.535	0.814	0.814	0.837
	H_E	0.860	0.748	0.912	0.894	0.804	0.589	0.875	0.819	0.802
	P	0.897	0.0167*	0.131	0.421	0.393	0.536	0.471	0.944	0.996
Kd	H_O	0.860	0.628	0.860	0.907	0.698	0.535	0.814	0.814	0.837
	H_E	0.860	0.748	0.912	0.894	0.804	0.589	0.875	0.819	0.802
	P	0.897	0.0167*	0.131	0.421	0.393	0.536	0.471	0.944	0.996
La	H_O	0.895	0.486	0.947	0.895	0.737	0.579	0.947	0.737	0.737
	H_E	0.880	0.621	0.916	0.865	0.763	0.569	0.884	0.724	0.760
	P	0.910	0.005*	0.761	0.918	0.861	0.732	0.839	0.900	0.886
Na	H_O	0.829	0.600	0.886	0.914	0.514	0.457	0.800	0.686	0.714
	H_E	0.812	0.607	0.925	0.926	0.602	0.506	0.793	0.790	0.844
	P	0.898	0.562	0.357	0.512	0.001*	0.240	0.291	0.749	0.290
Ad	H_O	0.975	0.861	0.875	0.875	0.775	0.725	0.925	0.625	0.800
	H_E	0.864	0.806	0.908	0.892	0.745	0.705	0.896	0.774	0.760
	P	0.750	0.848	0.940	0.625	0.715	0.816	0.622	0.192	0.571
Me	H_O	0.810	0.721	0.857	0.907	0.651	0.581	0.860	0.791	0.605
	H_E	0.848	0.797	0.901	0.908	0.724	0.667	0.857	0.798	0.782
	P	0.545	0.228	0.187	0.316	0.054	0.636	0.685	0.160	0.058
Md	H_O	0.800	0.821	0.975	0.875	0.825	0.525	0.825	0.625	0.775
	H_E	0.822	0.728	0.930	0.865	0.735	0.605	0.870	0.752	0.797
	P	0.014*	0.176	0.034*	0.691	0.470	0.391	0.750	0.065	0.483
Mc	H_O	0.816	0.526	0.921	0.816	0.684	0.658	0.868	0.842	0.711
	H_E	0.833	0.607	0.861	0.895	0.735	0.663	0.862	0.822	0.806
	P	0.844	0.229	0.612	0.228	0.097	0.754	0.556	0.982	0.127
Mb	H_O	0.795	0.682	0.841	0.841	0.750	0.818	0.818	0.841	0.750
	H_E	0.804	0.730	0.922	0.902	0.740	0.718	0.844	0.767	0.788
	P	0.348	0.628	0.025*	0.073	0.414	0.520	0.718	0.993	0.790
Ma	H_O	0.825	0.600	0.925	0.975	0.750	0.725	0.875	0.800	0.800
	H_E	0.806	0.616	0.929	0.920	0.708	0.666	0.821	0.793	0.788
	P	0.874	0.157	0.604	0.455	0.529	0.814	0.378	0.276	0.474
Ae	H_O	0.806	0.500	0.800	0.889	0.639	0.667	0.778	0.778	0.639
	H_E	0.813	0.630	0.933	0.916	0.638	0.663	0.842	0.810	0.777
	P	0.510	0.297	0.003*	0.634	0.710	0.414	0.366	0.646	0.037*

SUPPLEMENTARY TABLES FOR CHAPTER 5

Table S1. Coastal transect locality population means ($\overline{X}$) and variances (σ^2) for all quantitative traits characters. Adjusted values for body dimensions are given. See text for key to abbreviations.

	A01		A02		A03		A04		A05		A06		A07		A08	
	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2
SVL	71.8100	8.3400	76.4500	7.4800	77.0600	5.2800	71.7900	7.3100	72.5100	13.7000	68.9000	5.0300	70.9600	6.6100	69.4700	8.5500
JL	0.9270	0.0020	0.9457	0.0010	0.9293	0.0000	0.9210	0.0010	0.9189	0.0010	0.9260	0.0020	0.9345	0.0010	0.9329	0.0010
HL	1.3292	0.0010	1.3304	0.0010	1.3201	0.0000	1.3122	0.0000	1.3102	0.0000	1.3159	0.0010	1.3236	0.0000	1.3149	0.0010
HD	-0.0806	0.0010	-0.0714	0.0010	-0.1011	0.0020	-0.1066	0.0020	-0.0452	0.0010	-0.0145	0.0030	-0.0393	0.0020	-0.0370	0.0010
HW	0.2425	0.0010	0.2348	0.0010	0.2280	0.0010	0.2285	0.0020	0.2978	0.0010	0.2781	0.0020	0.2739	0.0010	0.2744	0.0010
ULL	1.1339	0.0020	1.1816	0.0010	1.1259	0.0010	1.1339	0.0010	1.0285	0.0670	1.1263	0.0050	1.1448	0.0010	1.1249	0.0020
LLL	0.8355	0.0010	0.8391	0.0010	0.8158	0.0020	0.8335	0.0010	0.8373	0.0010	0.8360	0.0010	0.8403	0.0010	0.8448	0.0010
DLL	1.3367	0.5040	1.5839	0.3150	1.7349	0.0020	1.7220	0.0010	1.6005	0.0040	1.5944	0.0070	1.6367	0.0040	1.6140	0.0030
DSC	76.2000	54.6220	77.2000	22.4000	72.3000	20.4560	77.1000	17.4330	69.6000	26.9330	75.5000	70.0560	76.5000	29.8330	76.5000	22.0560
VSC	78.5000	12.7220	78.6000	8.0440	74.6000	21.3780	76.8000	28.6220	76.0000	32.0000	73.9000	38.9890	82.7000	30.4560	79.4000	17.1560
PSC	8.9000	0.9890	8.7000	0.6780	9.3000	0.9000	9.5000	0.7220	8.4000	0.9330	8.1000	0.5440	8.3000	0.4560	8.5000	1.1670
SSC	0.9000	0.3220	1.7000	0.9000	1.1000	0.3220	1.3000	0.4560	1.3000	0.6780	0.8000	1.9560	1.1000	1.2110	1.3000	0.4560
LPH	0.0000	0.0000	0.1000	0.1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
LPAB	2.6000	11.1560	1.9000	5.6560	1.5000	10.5000	2.7000	13.3440	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
LPPB	1.4000	4.2670	1.5000	4.0560	0.5000	1.1670	2.5000	17.8330	0.0000	0.0000	0.1000	0.1000	0.0000	0.0000	0.0000	0.0000
HEAD	3.4000	0.9330	2.9000	1.6560	2.0000	1.1110	1.1000	1.2110	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.4000	1.6000
CLOAK	2.3000	1.3440	2.2000	0.8440	1.5000	0.5000	1.1000	0.5440	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
RED	0.3689	0.0070	0.3453	0.0020	0.3541	0.0030	0.3471	0.0030	0.3806	0.0010	0.4083	0.0040	0.4062	0.0000	0.4195	0.0000
GREEN	0.4607	0.0030	0.4362	0.0010	0.4261	0.0040	0.4084	0.0030	0.3353	0.0000	0.3517	0.0010	0.3296	0.0000	0.3361	0.0010

Table S2. Transitional transect locality population means ($\overline{X}$) and variances (σ^2) for all quantitative traits characters. Adjusted values for body dimensions are given. See text for key to abbreviations.

	F01		F02		F03		F04		F05		F06		F07		F08	
	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2	$\overline{X}$	σ^2
SVL	75.1700	3.9700	72.7900	9.9400	76.0800	4.8400	79.0300	6.6500	72.2500	7.3100	75.9700	11.8100	77.5400	6.3100	75.8800	15.5600
JL	1.1716	0.0002	1.1548	0.0003	1.1617	0.0002	1.1552	0.0001	1.1734	0.0001	1.1668	0.0001	1.1685	0.0003	1.1635	0.0001
HL	1.3203	0.0001	1.3156	0.0001	1.3141	0.0001	1.3139	0.0001	1.3231	0.0001	1.3179	0.0000	1.3234	0.0002	1.3178	0.0001
HD	0.8740	0.0000	0.8587	0.0004	0.8636	0.0002	0.8745	0.0001	0.8723	0.0003	0.8907	0.0004	0.8966	0.0003	0.8822	0.0001
HW	1.0227	0.0003	1.0061	0.0004	1.0180	0.0001	1.0303	0.0002	1.0232	0.0003	1.0401	0.0002	1.0464	0.0001	1.0293	0.0002
ULL	1.2955	0.0003	1.2775	0.0004	1.2855	0.0001	1.2933	0.0002	1.2798	0.0002	1.2930	0.0003	1.2910	0.0003	1.2947	0.0003
LLL	1.2381	0.0005	1.2364	0.0003	1.2207	0.0003	1.2357	0.0003	1.2336	0.0001	1.2308	0.0003	1.2299	0.0001	1.2358	0.0003
DLL	1.3943	0.0013	1.3956	0.0015	1.4159	0.0011	1.4391	0.0004	1.3925	0.0007	1.4094	0.0010	1.4092	0.0005	1.4145	0.0003
DSC	75.9000	27.6556	75.7000	45.1222	78.0000	14.8889	73.2000	39.9556	75.5000	31.1667	72.2000	35.2889	76.5000	10.2778	77.7000	59.1222
VSC	75.9000	13.8778	79.7000	24.4556	79.8000	12.1778	74.7000	18.2333	75.5000	35.8333	78.6000	17.1556	76.6000	16.7111	75.1000	18.3222
PSC	9.0000	0.8889	9.5000	0.7222	9.1000	0.7667	9.4000	0.9333	9.4000	0.7111	9.4000	0.7111	9.1000	0.9889	9.1000	0.9889
SSC	1.1000	0.7667	1.4000	0.4889	1.2000	0.6222	1.2000	0.1778	1.8000	0.8444	1.4000	0.4889	2.1000	1.6556	1.2000	0.1778
LPH	0.0000	0.0000	0.2000	0.4000	0.0000	0.0000	0.0000	0.0000	0.1000	0.1000	0.0000	0.0000	0.7000	3.5667	1.1000	0.7667
LPAB	7.1000	30.5444	11.4000	61.6000	6.5000	36.5000	3.9000	6.7667	6.4000	76.9333	0.4000	1.6000	8.2000	99.0667	12.9000	90.7667
LPPB	2.7000	12.4556	3.6000	9.3778	2.0000	8.0000	2.3000	7.7889	3.2000	17.7333	0.4000	1.6000	5.5000	62.0556	6.5000	41.8333
HEAD	2.6000	2.2667	1.6000	1.1556	2.5000	1.1667	0.7000	1.1222	1.0000	1.5556	0.3000	0.9000	0.4000	0.4889	0.6000	0.9333
CLOAK	2.2000	1.5111	1.1000	0.7667	1.8000	1.5111	0.5000	0.5000	1.1000	1.4333	0.4000	0.4889	0.5000	0.9444	0.1000	0.1000
RED	0.3888	0.0009	0.3860	0.0006	0.4043	0.0025	0.3968	0.0026	0.3728	0.0044	0.3680	0.0022	0.3751	0.0042	0.4406	0.0022
GREEN	0.5486	0.0026	0.5264	0.0025	0.5134	0.0027	0.5136	0.0016	0.5088	0.0032	0.3592	0.0002	0.4674	0.0059	0.5067	0.0040

Table S3. Pearson correlations for each character set of quantitative traits for the coastal transect. Upper triangular half shows scale count and marking pattern correlations, lower triangular half shows raw body dimension correlations. See text for key to abbreviations. * = significant at the 0.05 level. ** = significant at the 0.01 level (two-tailed test).

	VSC	DSC	SSC	LPA	LPB	HEAD	CLOAK	
		0.284*	-0.096	0.043	DSV	-	-	-	-
JL	0.767**		-0.105	0.076	VSC	-	-	-	-
HL	0.860**	0.830**		0.063	PSC	-	-	-	-
HD	0.571**	0.588**	00.544**		-0.05	-0.043	0.202	0.211	LPH
HW	0.655**	0.586**	0.537**	0.711**		0.642**	0.384**	0.442**	LPA
ULL	0.473**	0.403**	0.462**	0.230*	0.225*		0.339**	0.415**	LPB
LLL	0.780**	0.595**	0.706**	0.453**	0.476**	0.407**		0.836**	HEAD
DLL	0.692**	0.552**	0.649**	0.16	0.319**	.450**	0.496**		
	SVL	JL	HL	HD	HW	ULL	LLL	

Table S4. Pearson correlations for each character set of quantitative traits for the transitional forest transect. Upper triangular half shows scale count and marking pattern correlations, lower triangular half shows raw body dimension correlations. See text for key to abbreviations. * = significant at the 0.05 level. ** = significant at the 0.01 level (two-tailed test).

	VSC	DSC	SSC	LPA	LPB	HEAD	CLOAK	
		0.376**	0.015	0.01	Dsc	-	-	-	-
JL	0.687**		0.144	0.018	Vsc	-	-	-	-
HL	0.822**	0.792**		0.11	Pmsc	-	-	-	-
HD	0.714**	0.664**	0.679**		0.595**	0.714**	-0.172	-0.122	LPH
HW	0.779**	0.633**	0.702**	0.858**		0.840**	0.185	0.081	LPA
ULL	0.755**	0.633**	0.679**	0.596**	0.663**		0.083	0.038	LPB
LLL	0.677**	0.473**	0.626**	0.443**	0.512**	0.665**		0.831**	HEAD
DLL	0.536**	0.236*	0.387**	0.307**	0.369**	0.469**	0.340**		
	SVL	JL	HL	HD	HW	ULL	LLL	

Table S5. Principal component matrix for markings and scale count data on the coastal transect

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
PSC	0.492	0.125	0.282	0.732	-0.354	0.006	0.042
SSC	0.073	-0.203	0.937	-0.260	0.062	0.071	0
LPH	0.203	0.789	0.135	0.145	0.544	-0.019	0.003
LPA	0.734	-0.452	-0.011	0.067	0.259	-0.429	0
LPP	0.701	-0.445	-0.173	0.140	0.323	0.395	0.025
HEAD	0.807	0.280	-0.081	-0.389	-0.214	0.002	0.259
CLOAK	0.869	0.243	-0.047	-0.234	-0.214	0.037	-0.284
Eigen value	2.727	1.219	1.014	0.855	0.688	0.347	0.150
% variance	38.950	17.420	14.480	12.210	9.830	4.950	2.160

Table S6. The first (un-standardised) canonical function coefficient for the coastal transect.

Coastal transect	
Variable	
HW	-0.504
RED	-0.716
GREEN	0.808
PC1	0.631
Eigen Value	6.889

Table S7. Principal component matrix for bioclimatic data on the coastal transect.

Variable	PC1	PC2
Annual mean temperature	0.956	0.051
Altitude	0.334	-0.644
Annual precipitation	-0.944	0.199
Habitat	-0.058	0.900
Precipitation seasonality	0.875	0.464
Eigen value	2.687	1.482
% Variation	53.740	29.640

Table S8. Principal component matrix for markings and scale count data on the transitional forest transect

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
PSC	0.220	-0.176	0.676	-0.677	0.068	0.022	0
SSC	0.079	-0.266	0.713	0.641	-0.043	0.035	0.020
LPH	0.830	-0.216	-0.172	0.110	0.456	0.123	0.018
LPA	0.905	0.136	-0.066	-0.014	-0.328	0.135	-0.177
LPP	0.952	0.029	-0.015	0.019	-0.100	-0.25	0.143
HEAD	0.064	0.958	0.090	-0.011	-0.054	0.157	0.207
CLOAK	0.029	0.921	0.211	0.094	0.216	-0.132	-0.184
Eigen value	2.473	1.933	1.052	0.891	0.382	0.140	0.130
% variance	35.330	27.610	15.030	12.730	5.450	1.850	1.990

Table S9. The first (un-standardised) canonical function coefficient for the transitional forest transect.

Transitional forest transect	
Variable	
HD	-5.770
GREEN	0.713
PC2	0.622
Eigen Value	2.431

Table S10. Principal component matrix for bioclimatic data on the transitional forest transect.

Variable	PC1	PC2
Annual mean temperature	0.988	0.090
altitude	-0.552	0.576
Annual precipitation	-0.980	-0.153
Habitat	0.428	-0.791
Precipitation seasonality	0.864	0.483
Eigen value	3.710	1.223
% Variation	63.420	24.460

Figure S1.

Coastal transect bioclimate

Figure S2.

Transitional forest transect bioclimate
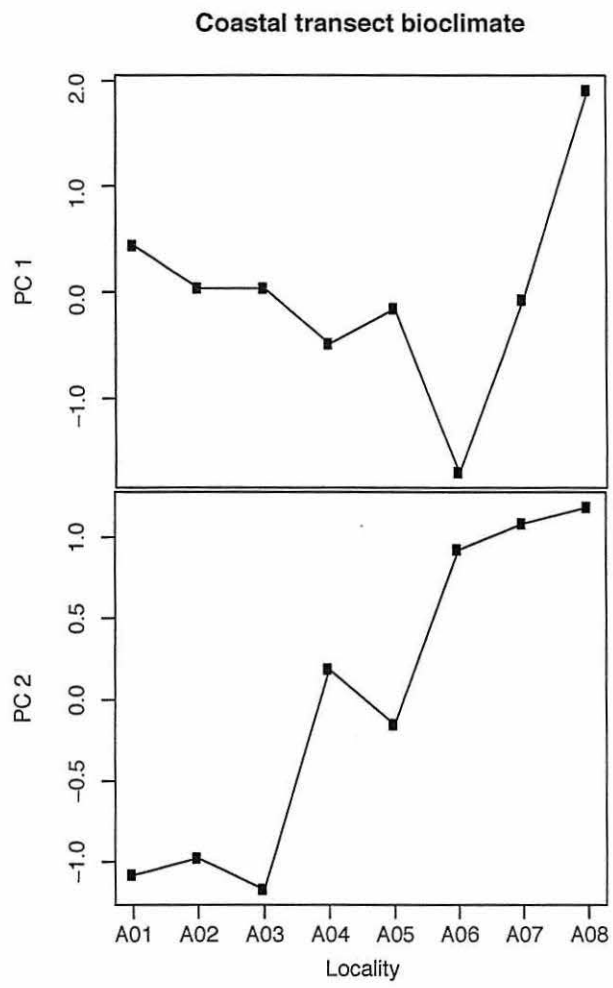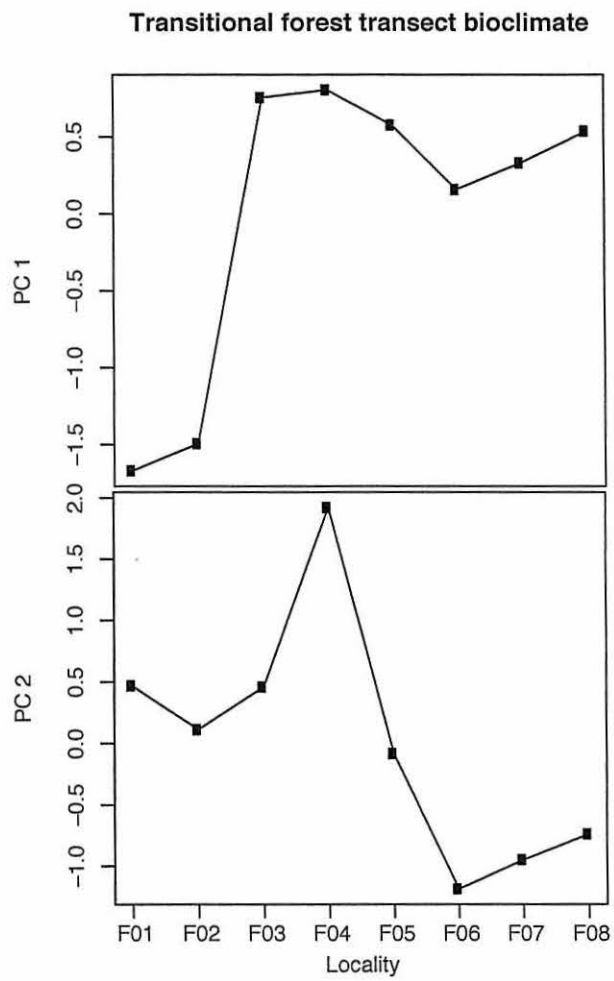
Supplementary Tables for Chapter 6

Table S1.

**Standardized canonical correlation
coefficient - Coastal transect**

UV1 anterior	1.042
Green anterior	2.092
Yellow/Orange anterior	-1.315
Red anterior	0.227
UV1 posterior	0.217
Blue posterior	-0.375
Yellow/Orange posterior	0.800
Red posterior	-0.971
CONSTANT	-15.445

Table S2.

**Standardized canonical correlation
coefficient - Transitional forest transect**

UV1 anterior	1.152
UV2 anterior	-2.100
Blue anterior	0.525
Green anterior	0.451
Red anterior	-0.377
UV1 posterior	1.294
UV2 posterior	-0.298
Blue posterior	1.931
Green posterior	2.234
Yellow/Orange posterior	0.976
Red posterior	2.305
CONSTANT	-64.166

APPENDIX D

PUBLICATIONS

PRIMER NOTE

Ten polymorphic tetranucleotide microsatellite markers isolated from the *Anolis roquet* series of Caribbean lizards

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Abstract

The *Anolis roquet* series of Caribbean lizards provides natural replicates with which to examine the role of historical contingency and ecological determinism in shaping evolutionary patterns. Here, we describe 10 polymorphic tetranucleotide microsatellites to facilitate studies on population differentiation and gene flow. All loci successfully amplified in several species from this series. Genotyping 96 individuals from two *A. roquet* populations demonstrated the markers' suitability as population genetic markers: genetic diversity was high (9–22 alleles per locus); there were no instances of linkage disequilibrium; and, with one exception, all genotypic frequencies conformed to Hardy–Weinberg equilibrium expectations.

Keywords: anole lizard, *Anolis roquet*, DNA, enrichment protocol, microsatellite

Received 31 January 2006; revision accepted 2 March 2006

The *Anolis roquet* series of tree lizard inhabits the southern Lesser Antillean Islands of the Caribbean. Geographical isolation over millions of years has resulted in inter-island speciation (Creer *et al.* 2001). Although most islands have only a solitary species and no island supports more than two, substantial intraspecies geographical variation in colour pattern has occurred within islands, driven by a combination of historical contingency and ecological determinism (Thorpe 2002; Thorpe & Stenson 2003). As such, the *A. roquet* series provides natural replicates with which to examine the role of historical divergence and selection in determining evolutionary patterns.

Polymorphic codominant markers additional to the eight existing microsatellites for *A. roquet* (Ogden *et al.* 2002) are now required to increase statistical power in analyses of population differentiation and gene flow within the species of this series. Here, we report the identification of 10 polymorphic microsatellite loci from the *A. roquet* series, each of which is amplifiable in multiple species from this group. Their suitability as population genetic markers is illustrated from *A. roquet* sample genotyping.

A genomic library was constructed and enriched for tetranucleotide microsatellite repeats for six *A. roquet* series

species (*A. roquet* from Martinique, *A. bonairensis* from Bonaire, *A. trinitatis* from St Vincent, *A. richardii* and *A. aeneus* from Grenada, and *A. extremus* from Barbados). Genomic DNA was extracted from autotomized tail tips stored in 100% ethanol using a QIAGEN DNeasy Tissue Kit according to the manufacturer's instructions for purification from rodent tails. For each species, 5 ng DNA was pooled from equal amounts of DNA from eight lizards, which had been sampled from different locations spread across the species geographical range. We then followed a modified enrichment technique developed by Gardner *et al.* (1999), which is based on magnetic/biotin capture of repetitive sequences from restricted DNA, with minor modifications:

1 After the addition of the magnetic bead mixture to the prepared DNA fragments, the beads were washed eight times in 100 μL of 1 × SSC (0.15 M NaCl, 15 mM trisodium citrate) with 10 pmol of linker oligo A (S61: 5'-GGCCAG-AGACCCCAAGCTTCG-3'). The first four washes were performed at 40 °C, the latter four at 50 °C.

2 Initially, four biotinylated oligos were used to enrich for AAAG, TCAG, TACA and TAGA repeat microsatellites in *A. bonairensis*, *A. trinitatis* and *A. roquet*. The success of isolating TAGA repeats in particular led to enrichment for this repeat only for the remaining species.

3 pCR2.1-TOPO vector and TOP10F' competent cells were used for cloning, according to TOPO TA Cloning Kit instructions (Invitrogen).

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Table 1 Polymorphic microsatellite loci for the *Anolis roquet* series. Locus name prefix indicates the species of origin: Aae, *A. aeneus*; Abo, *A. bonairensis*; Aex, *A. extremus*; Ari, *A. richardii*; Aro, *A. roquet*; Atr, *A. trinitatis*. The repeat motif and GenBank Accession number of the sequenced clone are given. Primer sequences, annealing temperature (T_a in °C) and MgCl$_2$ concentration (MgCl$_2$ (mM)) for optimal PCR amplification in the species of origin are given for each locus, alongside the number (N) and range (in base pairs) of alleles found among six specimens of the species of origin sampled from different localities. Number of alleles, allele size range, and expected (H_E) and observed (H_O) heterozygosities per locus averaged over two sampled populations are described for the eight microsatellites that were polymorphic within *A. roquet* ($n = 96$ from two disparate populations from Martinique)

Locus	Repeat motif	Primer sequence (5′–3′) (F, forward; R, reverse)	T_a (°C)	MgCl$_2$ (mM)	Species of origin screening ($n = 6$)		*A. roquet* screening ($n = 96$)				GenBank Accession no.
					N	Allele size range (bp)	N	Allele size range (bp)	H_E (± SD)	H_O (± SD)	
AaeP2F9	(CTAT)$_{13}$	F: CAATGTTTTGCTCTTGCTATTT* R: GGCTGATTTGTCCTTTCTGG	55	2.5	5	219–243	16	223–281	0.86 (± 0.00)	0.89 (± 0.03)	DQ379371
AaeP2F5	(CTAT)$_8$	F: GCAAAGGCAATAGGAAAAGG* R: GTTGGCGATGTCCCATAAAC	55	1.5	9	268–326	15	272–352	0.86 (± 0.00)	0.84 (0.03)	DQ379372
AboP4A9	(CTAT)$_9$	F: GTGACTATGAAGGGGAATCTTG* R: GATGTAGGCTTTGCTGCTGT	55	1.5	4	359–371	12	335–365	0.51 (± 0.24)	0.46 (0.23)	DQ379373
AexP2E3	(CTAT)$_{13}$(AC)$_8$	F: TCTTCCTCCCTTTCCCAGAT* R: TAGCTTCCCCTTTTGCTTTG	55	2.5	8	207–257	18	211–263	0.86 (± 0.02)	0.78 (0.13)	DQ379374
AexP1H11	(CTAT)$_{11}$	F: GCTATCCATCCATCATTTCTATGT* R: AAACTGTAATTCCCAAGATTCCA	50	3.5	7	273–303	20	249–301	0.91 (± 0.01)	0.83 (0.08)	DQ379375
AexP4H6	(CT/CAT)$_{17}$	F: TCTGGGTTTTCTGGAAGCTG* R: TCAAACCATGTAGGAACCTGTG	53	3.5	7	167–217	22	171–231	0.90 (± 0.01)	0.74 (0.17)	DQ379376
AriP2D8	(CT/CAT)$_{24}$	F: GGAGCAGAAAGAGAAGAAACATC* R: TCAAACGGGAAAACAAGAAC	53	3.5	3	227–307	NA	NA	NA	NA	DQ379377
AroHJ2	(TAGA)$_{10}$	F: ACATGAATGGTGGGAG* R: TTGACCACACTCTGATGTTGC	60	1.5	4	218–226	9	210–242	0.77 (± 0.06)	0.70 (0.08)	DQ379378
AroHJ5	(TAGA)$_{11}$	F: TCTTGGAGAAAAGGCAGAAAG* R: CTGGAGGCCTACACTATGTCC	55	3	4	211–223	16	187–273	0.84 (± 0.00)	0.70 (0.05)	DQ379379
AtrP16.55	(CTAT)$_6$CAT (CTAT)$_{12}$CGT(CTAT)$_6$	F: GATAGTGGGCTGGGGAGAG* R: CCCGCTCCTGAGATAGATTG	50	3.5	11	97–149	NA	NA	NA	NA	DQ379380

*Fluorescent dye-labelled primer (CY5 or CY5.5 dye).

Table 2 Cross-species amplification of 10 microsatellite primer pairs within the *Anolis roquet* series. Two samples per species were screened for PCR amplification of a well-defined band in the expected size range (+, presence; –, absence), using conditions listed in Table 1 and visualized on 2% agarose. Additional PCR optimization may recover loci not shown to amplify here

Locus	Anoles						
	A. aeneus	*A. bonairensis*	*A. extremus*	*A. griseus*	*A. richardii*	*A. roquet*	*A. trinitatis*
AaeP2F9	+	–	+	+	+	+	+
AaeP2F5	+	–	+	+	+	+	+
AaeP2E3	–	–	+	+	–	+	+
AboP4A9	–	+	–	+	+	+	–
AexP1H11	+	+	+	+	–	+	–
AexP4H6	+	+	+	+	+	+	–
AriP2D8	+	+	+	+	+	+*	+
AroHJ2	–	+	+	–	+	+	–
AroHJ5	+	+	+	+	+	+	+
AtrP16.55	+	+	+	+	+	–	+

*Some genotyped samples (see Table 1) have an ambiguous banding pattern.

4 For the detection of microsatellite-containing clones using polymerase chain reaction (PCR), we used Promega PCR buffer and *Taq* DNA polymerase with M13 forward (–20: 5′-GTAAAACGACGGCCAG-3′) and M13 reverse (5′-CAGGAAACAGCTATGAC-3′) primers, running the same PCR program used to amplify the captured DNA fragments.

Purified PCR products of 144 cloned inserts likely to contain a microsatellite were then sequenced by MWG-Biotech. Ninety-three of these contained a microsatellite (≥ 5 repeats). Some, however, were duplicates ($n = 16$) or had insufficient flanking region to enable primer design ($n = 23$). Fifty-five microsatellites were deemed unique and possessed adequate flanking regions for primer design, which was performed using PRIMER 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

Primers were tested on six individuals of the species from which the cloned insert was derived. Loci were amplified using 5 ng of template DNA, 1.5–3.5 mM $MgCl_2$ (Table 1), 0.2 mM of each dNTP, 0.5 µM of each primer (forward labelled with CY5 or CY5.5 dye), 0.5 U of *Taq* DNA polymerase and 1× buffer (Promega) in 10 µL. PCR was performed using a profile of denaturation for 2 min at 94 °C, followed by 30 thermal cycles (30 s at 94 °C, 30 s at a locus-specific annealing temperature [Table 1], and 30 s at 72 °C) and a final extension period of 5 min at 72 °C. A total of 10 loci yielded reproducible, easily interpreted polymorphic bands when analysed on a CEQ 8000 Genetic Analysis System (Beckman Coulter), with CEQ DNA Size Standard Kit-400 used as an internal size standard (Table 1).

To illustrate the utility of this microsatellite bank for *A. roquet* series population genetic studies, we screened eight

A. roquet from three phylogenetic lineages (Thorpe & Stenson 2003). We then genotyped 96 *A. roquet* from two disparate populations at the eight loci that yielded reproducible, easily interpreted polymorphic bands for this species (Table 1). Genetic diversity was high: the number of alleles per locus ranged from nine to 22 (mean = 16; SD ± 4); gene diversity and observed heterozygosity per locus averaged over the two samples ranged from 0.51 to 0.91 (mean = 0.81; SD ± 0.13) and from 0.46 to 0.89 (mean = 0.74; SD ± 0.13), respectively. Tested in GENEPOP version 3.3 (Raymond & Rousset 1995), there were no significant genotypic linkage disequilibria among pairs of loci and all genotypic frequencies conformed to Hardy–Weinberg equilibrium expectations ($P > 0.05$, after Bonferroni correction), with the exception of a heterozygote deficit at locus AexP4H6 in population E1. F_{ST} estimates between the populations for each locus ranged from 0.01 and 0.45, and yielded a highly significant ($P < 10^{-5}$) global F_{ST} estimate of 0.10, suggesting that restricted gene flow and genetic drift are important determinants of genetic structure. Given the amplification of all 10 loci in multiple species from the *A. roquet* series (Table 2), this suite of markers serves not only to resolve patterns of gene flow within *A. roquet* but also enables the initiation of new genetic studies across the *A. roquet* series.

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Development of microsatellite markers in the St Lucia anole, *Anolis luciae*

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Abstract

Anolis lizards are important models in studies of ecology and evolution. Here we describe 13 polymorphic microsatellites for use in population screening in the St Lucia anole, *Anolis luciae*, that can be used as a natural replicate to *Anolis roquet* on Martinique to study processes involved in population differentiation and speciation. Genotyping of 32 individuals using M13 tails and FAM-labelled universal M13 primers showed that all loci were polymorphic with high genetic diversity, averaging at 16.8 alleles per locus. Genotypic frequencies conformed to Hardy–Weinberg expectations, and there were no instances of linkage disequilibrium between loci.

Keywords: *Anolis luciae*, Caribbean anole, DNA, M13 tail, microsatellite, pigtail

Received 18 January 2008; revision accepted 9 March 2008

Anolis lizards have been extensively studied in the Caribbean and have had a major influence in the development of evolutionary ecology theory (Losos in press). Recently, studies on the Martinique anole, *Anolis roquet*, have highlighted the usefulness of Lesser Antillean species in the study of speciation (Ogden & Thorpe 2002; Thorpe 2005). The complex geological history and environment observed on Martinique allows for the study of allopatric effects and ecological determinism in population differentiation and speciation. Saint Lucia, adjacent to Martinique, is another Lesser Antillean island with complex geological history and similar environmental variation to Martinique. The endemic anole, *Anolis luciae*, is closely related to *A. roquet* (Thorpe & Stenson 2003) and can be used as a natural replicate to generalize observations from Martinique to shed light on aspects of speciation and parallel evolution in Lesser Antillean anoles. Here, we report 13 microsatellite loci for use in *A. luciae*. One (ABO-P4A9) is cross-amplified from the related *Anolis bonairensis* (Gow *et al.* 2006) while the remaining 12 are new and developed from *A. luciae*.

DNA samples were extracted from automotized tail tips collected from different locations across the species' geographical range. DNA samples were used to construct

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a genomic library enriched for tetranucleotide microsatellite repeats according to the protocol developed by Gardner *et al.* (1999) and modified by Gow *et al.* (2006). Purified polymerase chain reaction (PCR) products from 134 clones potentially containing microsatellites were sequenced by Macrogen (www.macrogen.com). From these clones, 27 had unique microsatellite motifs and had adequate flanking regions that were suitable for primer design using the program PRIMER 3 (Rozen & Skaletsky 1998).

An M13 tail was added to the forward primers allowing the use of a FAM-labelled universal M13 primer for amplification (Oetting *et al.* 1995). Reverse primers were pigtailed at the 5′ end (GTTT) to reduce stutter and improve reliability of allele scoring (Brownstein *et al.* 1996). Primers were first tested on four different individuals. Loci were amplified using 5 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.05 μM of the forward primer, 0.5 mM of the reverse primer and of the labelled primer, and 0.5 U of *Taq* DNA polymerase with associated 5× buffer (Promega). PCR was performed with a denaturation time of 2 min at 95 °C, followed by 29 cycles (30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C) and a final extension period of 5 min at 72 °C. Samples were run on an ABI 3130xl genetic analyser with the internal size standard LIZ-600 and analysed with the software GENEMAPPER version 4.0 (Applied Biosystems). Thirteen loci amplified and showed variation in the first four samples, and were screened on a further 32 individuals from one *A. luciae* population (Mon Repos, UTM coordinates

Table 1 Polymorphic microsatellite loci for *Anolis luciae*. Locus name prefix indicates the species of origin: Abo, *Anolis bonairensis*; Alu, *A. luciae*. Final PCR conditions for all primers were: 1.5 mM $MgCl_2$, 55 °C annealing temperature. Primer name, repeat motif and GenBank Accession number of the sequenced clone are given alongside allele size range, number of alleles (N), observed (H_O) and expected (H_E) heterozygosities for the 13 microsatellites that were polymorphic within *A. luciae* ($n = 32$ from a single population from St Lucia)

Primer name	Repeat	Primer sequence (F, forward; R, reverse)	Allele size range	N	H_O	H_E	GenBank Accession no.
ABO-P4A9	$(CTAT)_9$	F: GTGACTATGAAGGGGAATCTTG	347–407	15	0.88	0.88	DQ379373
		R: GTTTGATGTAGGCTTTGCTGCTGT					
ALU-MS02	$(AAAG)_{16}$	F: GAAATGCAGCTTCGATCACA	177–316	21	0.78	0.94	EU379658
		R: GTTTATTGGGAGAAGTGGGTTGC					
ALU-MS04	$(AAAG)_{15}$	F: TCAGTCTAAGGGTGGGAGGA	272–327	14	0.84	0.89	EU379659
		R: GTTTGCTCATTAGGATTTGGGACTT					
ALU-MS06	$(TAGA)_{10}$	F: CCTGATGCGCACAAAGAATA	240–284	12	0.87	0.86	EU379660
		R: GTTTTCAAGTCTGGCAATGGA					
ALU-MS10	$(AAAG)_8$	F: GGCTCTTGGCACCTGATAAA	252–351	10	0.78	0.82	EU379661
		R: GTTTCCAATCCTGGCAAAACATCT					
ALU-MS12	$(TACA)_5$	F: TACATACACCGTTGCCCACA	127–151	7	0.56	0.58	EU379662
		R: GTTTATCAGCACACACCAGTCAGC					
ALU-P8A3	$(AAAG)_{13}$	F: GCTGGAAAGATTAACAAAGATGG	213–268	17	0.78	0.89	EU379663
		R: GTTTCCCAACAAAAAGGATTCTGAC					
ALU-P8B10	$(AAAG)_{10}$	F: CAGAGAGTTCAAAAGGAATTGTCC	135–176	28	0.88	0.95	EU379664
		R: GTTTACTGCCTTTCCCTTATGGTC					
ALU-P8C7	$(GT)_7$	F: TCAATGAATGGGCTGGTGT	194–312	14	0.81	0.83	EU379665
		R: GTTTGGAAAGTGTTTCGCTTGA					
ALU-P8C9	$(AAAG)_{17}$	F: TCACTAAATGCCTCTAAGCTATTG	217–274	16	0.81	0.91	EU379666
		R: GTTTCTCCCAAAGGCAAGGTTTC					
ALU-P8E12	$(AAAG)_{15}$	F: TCCTGGACCCATGTGAAAAG	91–125	21	0.94	0.92	EU379667
		R: GTTTAAACAGGAGGCGAAGTTGG					
ALU-P8H7	$(AAAG)_{11}$	F: GGGGGTTCTGTGAATTGTTG	98–152	17	0.94	0.92	EU379668
		R: GTTTCCAAGGTATTCTTCCATTTGC					
ALU-P8H8	$(AAAG)_{12}$	F: GGCATCTCCATTTTAACAAGAAAG	111–190	26	0.94	0.96	EU379669
		R: GTTTGACAGATTTTCCTAGTTCCTCCTG					

easting 726279, northing 1533577, zone 20N). We also tested the utility in this species of the 10 loci previously isolated from different species of the *roquet* series. For these loci, the PCR conditions were described in Gow *et al.* (2006) and one of these (ABO-P4A9) amplified reliably in *A. luciae*.

In total, 13 loci amplified reliably, were polymorphic and did not show any evidence of null alleles. Allelic diversity was calculated for each locus, and ARLEQUIN version 3.01 (Schneider *et al.* 2000) was used to test for departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) (Table 1). Genetic diversity was high: number of alleles per locus ranged from seven to 28 and averaged 16.8 across all loci; mean expected heterozygosity was 0.87 with locus-specific values varying from 0.58 to 0.96. There were no significant departures from HWE, and none of the possible locus combinations showed significant genotypic LD, following sequential Bonferroni correction (Rice 1989). Extreme length variation is evident in two loci, ALU-MS02 and ALU-P8C7. However, there is no deviation from HWE in these loci and the 'outlying' alleles were found in different individuals. Thus, we are confident they are not artefacts.

Furthermore, we have just studied a limited number of individuals in one population. Some microsatellite loci in the closely related anole *A. roquet* have more than 80 alleles across the entire distribution range. Thus, we expect to find alleles with intermediate sizes when scoring more individuals from different populations. Microsatellite loci in the Lesser Antillean anole, *A. luciae*, hence show comparative levels of genetic diversity to other Lesser Antillean anoles (Ogden *et al.* 2002; Gow *et al.* 2006), and can be used for intra-island population genetic screening.

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The relative importance of ecology and geographic isolation for speciation in anoles

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The biogeographic patterns in sexually reproducing animals in island archipelagos may be interpreted as reflecting the importance of allopatric speciation. However, as the forms are allopatric, their reproductive isolation is largely untestable. A historical perspective integrating geology and molecular phylogeny reveals specific cases where ancient precursor islands coalesce, which allows the application of population genetics to critically test genetic isolation. The *Anolis* populations on Martinique in the Lesser Antilles are one such case where species-level populations on ancient precursor islands (*ca* 6–8 Myr BP) have met relatively recently. The distribution of the mtDNA lineages is tightly linked to the precursor island, but the population genetic analysis of microsatellite variation in large samples shows no evidence of restricted genetic exchange between these forms in secondary contact. This tests, and rejects, the hypothesis of simple allopatric speciation in these forms. By contrast, Martinique has pronounced environmental zonation, to which anoles are known to adapt. The population genetic analysis shows restricted genetic exchange across the ecotone between xeric coastal habitat and montane rainforest. This does not indicate full ecological speciation in these forms, but it does suggest the relative importance of the role of ecology in speciation in general.

Keywords: allopatric speciation; ecological speciation; Martinique anoles; Bayesian assignment

1. INTRODUCTION

The relative importance of the various factors that may potentially influence speciation in sexually reproducing animals (e.g. drift in spatial isolation, natural selection and sexual selection) is one of the central issues in the active debate on speciation (Claridge *et al.* 1997; Howard & Berlocher 1997; Smith *et al.* 1997; Magurran & May 1998; Orr & Smith 1998; Dieckmann & Doebeli 1999; Schneider *et al.* 1999; Schluter 2000; Barton 2001; Thorpe & Richard 2001; Ogden & Thorpe 2002; Doebeli & Dieckmann 2003; Gavrilets 2003; Tautz 2003; Dieckmann *et al.* 2004; Ortiz-Barrientos & Kane 2007; Ritchie 2007; Abbott *et al.* 2008). The traditional view of speciation in sexually reproducing animals is that it generally involves the divergence of populations over time while they are geographically apart (geographical or allopatric speciation; Mayr 1970). This approach is typified in genera on many island archipelagos where each island or island bank tends to have its own nominal species (Stenson *et al.* 2004; Ricklefs & Bermingham 2007), and generally speciation is assumed to occur when an island is colonized by dispersal from another; the subsequent geographical isolation allowing the founding population to evolve into a new species (see also Comes *et al.* 2008).

There are two conceptually different empirical approaches when seeking an insight into causative factors in evolutionary ecology, which are pertinent to

the question of speciation in these island archipelagos. One approach is to interpret the overall biogeographic or phylogenetic pattern (Losos & Schluter 2000; Borsa *et al.* 2007; Hyde & Vetter 2007) in order to deduce the population-level process that caused it. The other is to take a more reductionist stance and try and look in detail directly at the population-level processes that may have led to such a biogeographic or phylogenetic pattern. Examples of both approaches can be found using island anoles where both biogeographic patterns (Losos & Schluter 2000) and population-level approaches (Ogden & Thorpe 2002) have been employed in studies of speciation and ecology (reviewed in Thorpe 2005). Both approaches have their merits and difficulties when applied to island speciation studies. One difficulty with a biogeographic approach is that it is possible to regard the islands that are observed today as the entity to study, when, in fact, general sea-level changes, sea-floor uplifts in specific areas and volcanic activity may substantially alter the number and size of islands and their connectivity. A further difficulty is that without sound fossil evidence, we may have an inadequate or misleading view of the past distribution of species and their environmental conditions. Another critically important limitation is that with allopatric species, such as are found in island archipelagos, one may be dealing with just arbitrary nominal species making some biological interpretations tenuous or inappropriate. What we mean by speciation depends on what we mean by species (Wiens 2004). The term species, of course, encompasses the Linnaean name, the taxonomic category and potentially a biological entity, however defined by the species concept of choice. When we talk about speciation we

mean the origin of the biological entity and not the former two uses. Geographical variation within species is ubiquitous and population differentiation is not speciation (Magurran 1998). Hence, if allopatric populations are recognized as arbitrary nominal species on the basis of trivial population differentiation, then the process of 'speciation' can mean something different from the process that leads to, say, reproductive isolation between sympatric forms (see Mallet 2008). Consequently, patterns of speciation in arbitrarily recognized (nominal) allopatric species may tell us something about population differentiation, but they may have limited usefulness when trying to generalize to speciation involving reproductive isolation.

2. THE AREA AND THE ORGANISM

Anoles are small insectivorous, generally arboreal, iguanine lizards. Males have a dewlap (an extendable throat flap) that acts as a sexual signal and (in multi-species sympatric communities) species recognition (Losos 1985). There are approximately 400 described species of *Anolis*, making it the largest genus of amniote and perhaps the largest monophyletic tetrapod genus (Losos & Thorpe 2004). In some organisms, inter-specific hybrids are common (Mallet 2008), but sympatric anoles generally appear to behave like good biological species. Anoles are well studied, and these studies have influenced much of evolutionary ecology, including niche theory, adaptive radiation, behaviour, natural selection, biogeography and phylogeny (extensively reviewed by Losos in press). Their diversity and their focal role in many studies of evolutionary ecology make them good candidates for the study of speciation.

Approximately 150 Caribbean species are thought to have arisen from just two colonizations from the mainland (Jackman *et al.* 1997), so the number of species is due to diversification within the Caribbean rather than multiple colonization from the mainland. In the Greater Antilles islands of Jamaica, Cuba, Hispaniola and Puerto Rico, anoles live in communities with up to 11 species in sympatry. The molecular phylogenetic, morphological and ecological evidence provides an outstanding case of predictable adaptive radiation, where species with a specific morphology occupy specific niches, i.e. ecomorphs. This occurs independently, in parallel, on each Greater Antillean island (Losos in press).

By contrast, the Lesser Antillean anoles are either solitary or live in pairs. There are two main radiations (figure 1): the *roquet* series extend up to Martinique from the south and the *bimaculatus/wattsi* series extend down from the north to Dominica. Two species of the *roquet* series (one large and another small) are sympatric on St Vincent and Grenada (and Grenadines), and all other southern islands have solitary anoles. In the north, no *bimaculatus* (medium or large) series live sympatrically with other members of the series, but they can live in sympatry (species pairs) with anoles from the *wattsi* group. This results in one large and one small species on the Antigua/Barbuda bank, the St Martin bank and the St Kitts/Nevis bank, and solitary *bimaculatus* anoles on the remaining northern islands.
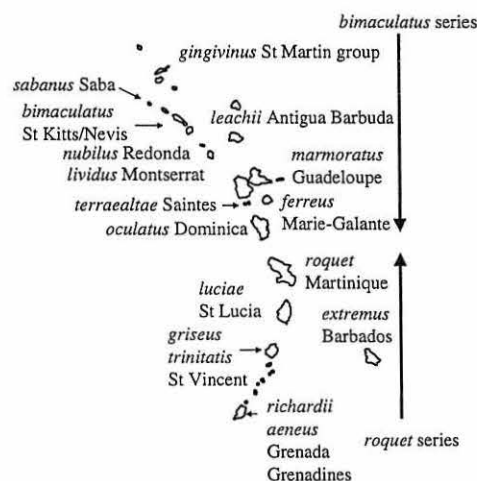
Figure 1. Lesser Antillean anoles. Distribution of the *A. roquet* series species in the south and the *A. bimaculatus* series in the north (*A. wattsi* group not shown).

This presents some clear biogeographic patterns. Each island or island bank has its endemic anole species. There are no empty islands as every single island and habitable islet has anoles. There is limited sympatry, but the anoles from the *roquet* and *bimaculatus* series are never sympatric with one another, their distributions do not even interdigitate.

Estimates of island-wide population size are going to be error prone; the extrapolation from biomass estimates on Dominica (Malhotra & Thorpe 1991) suggests a standing (breeding) population of 200 million anole adults (or more) and as females may produce an egg every two weeks, there may be a turnover of approximately a billion (or more) per year. Hence, while populations on small islands may often be thought of as fragile, it appears that anole populations may be very large and robust on small islands. This is compatible with the observations that currently no island or habitable islet in the Lesser Antilles is without anoles, and they may withstand even cataclysmic volcanic activity (Malhotra & Thorpe 2000). Consequently, this suggests that anole populations may increase rapidly in size after the founding colonization, and that, while bottlenecks may occur, they are not particularly vulnerable to prolonged bottlenecks after establishment.

The mountainous Lesser Antillean island possesses pronounced environmental zonation (Beard 1948). The Caribbean coastal strip (and the tip of southern and eastern peninsulas on some islands) may be hot and dry with seasonal rain and have xeric woodland. Higher elevation areas are cooler, with substantial, less seasonal, rain, with montane rainforest (giving way to cloud forest). The mid-Atlantic coast, which collects the warm moist trade winds, may have dense, wind-swept littoral woodland. The quantitative traits of anoles, such as scalation, body dimensions, hue and pattern, appear to be primarily genetically controlled when tested (Thorpe *et al.* 2005a; Calsbeek *et al.* 2006;
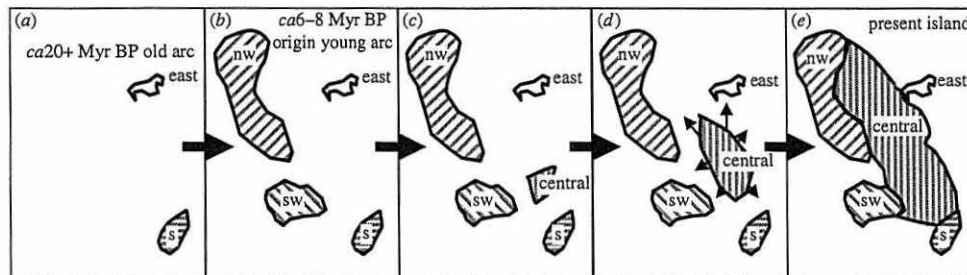
Figure 2. Geological history of the components of Martinique. Two islands in (*a*) the older arc, Caravelle (east) and St Anne (south (s)), were subsequently joined by a further two islands at the origin of (*b*) the younger arc, northwest (nw) and southwest (sw). General uplifting of (*c*) the central area, (*d*) continued until the central area joined the peripheral precursor islands into (*e*) the present single island of Martinique.

Calsbeek & Smith 2007), and show pronounced intraspecific geographical variation within islands in concert with this environmental zonation irrespective of phylogeographic lineage (Malhotra & Thorpe 2000; Thorpe *et al.* 2004). The overwhelming conclusion from studying field experiments on natural selection, correlations between the geographical variation in a trait and its putative cause, and parallels between patterns of geographical variation on independent islands, is that these patterns of ecotypic geographical variation are the result of rapid, pronounced and, in some instances, predictable, natural selection. Sexual selection may also play a role as visual sexual signals, such as male dewlap hue and sexually dimorphic elements of the hue and colour pattern, which may vary substantially with habitat types that have different ambient light conditions (Endler 1992; Leal & Fleishman 2004).

The biogeographic pattern of species distribution (figure 1) and molecular phylogeny suggests progressive overseas colonization from one island to another as in Thorpe *et al.* (2004), with endemic species evolving on each island or island bank. The *Anolis bimaculatus* and *wattsi* series come from the Greater Antilles in the north, and the *roquet* series from the mainland in the south. This overseas colonization by founders and subsequent speciation is a form of allopatric or geographical speciation, so the biogeographic pattern strongly reinforces the traditional view of the primary role of allopatric speciation in this type of organism. Some of the limitations of interpreting the pattern to deduce the cause, which are listed above, apply to this case. Allopatric speciation can only be said to have occurred if the island forms are more than just nominal species. As the current islands are spatially isolated, this is untestable. Here, another facet of the 'pattern' approach is pertinent, i.e. current islands do not represent the past islands. The case in point is Martinique, which is thought to be composed of precursor islands that have only recently coalesced into a single island. However, this may give us a rare opportunity to test, which is often not directly testable under natural conditions, the reproductive isolation of allopatric island populations and the impact of allopatry on speciation.

3. TESTING THE ROLES OF ALLOPATRY AND ECOLOGY IN SPECIATION
(a) *Martinique*
(i) *Island-wide geology and environment*
The island of Martinique in the mid-latitudes of the Lesser Antilles has been the single entity we recognize today for only a brief period of its geological existence (Maury *et al.* 1990; and references in Thorpe & Stenson 2003). For an extensive time during the period of the older arc, two islands existed: a southern island (St Anne) and an eastern island (Caravelle; figure 2*a*). The origin of the younger arc would have seen the eruption of two further islands, in the southwest (Trois Ilet) and northwest (figure 2*b*). The subsequent emergence of a central island (figure 2*c*) and the gradual uplifting of this region (figure 2*d*) eventually joined the four peripheral islands to form the single entity we know today as Martinique (figure 2*e*). Independent of this is the distinct environmental zonation typically found in mid-latitude, high-elevation, Lesser Antillean islands. There is a xeric strip on the Caribbean coast in the rain shadow of the major central mountains (figure 3), a xeric southern peninsula and a xeric eastern tip to the eastern Caravelle peninsula. The central mountains are largely covered with montane rainforest, with a sharp transition to xeric woodland on the Caribbean coast (figure 3). The middle section of the Atlantic coast has littoral conditions, but these change towards the north (more seasonal) and south (drier). The anoles appear to adapt by natural selection to these regimes, as tested in Thorpe & Stenson (2003) and figured in Thorpe (2005). Among other colour patterns, the Martinique anoles have zebra striping in these xeric regions, while those from montane rainforest have white (without UV) and black markings on a deeply saturated green background. Other quantitative traits, such as scalation and body proportion, also vary with habitat and these ecotypic patterns occur irrespective of lineage.

This system can be used to critically test the impact of allopatry on the potential for speciation, and relate this to the degree of genetic isolation associated with habitat differences (ecological speciation). This rests on two assumptions. First, the precursor islands need to be occupied while they were separate to give allopatric forms. Geologically recent colonization, after the
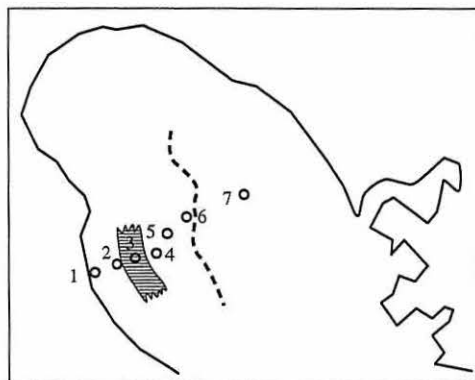
Figure 3. Secondary contact zone and ecotone in northwest Martinique. The dashed line marks the geological boundary between the northwest and central regions where populations on these precursor islands may make secondary contact. The cross-hatched area represents the ecotone where the xeric coastal habitat (transect localities 1 and 2) is in transition to the montane rainforest (transect localities 4–7).

islands had coalesced into the single island of Martinique, would not allow such a test. Second, if there were allopatric populations on these precursors, the timing and level of divergence needs to be compatible with that found in other species in the series and other series of Lesser Antillean anoles.

(ii) *Island-wide phylogeography*
Many of these questions can be answered by molecular phylogenetic and phylogeographic study. Tail tips were taken from 39 individuals from 15 localities for *A. extremus* and 68 individuals from 64 localities for *Anolis roquet*. *Anolis luciae* and *Anolis jacare* were the outgroup. Genomic DNA was extracted using the chelex method described in Surget-Groba *et al.* (2001) and a 1063 bp fragment of the cytochrome *b* gene was amplified with primers MTA-S (ATCT CAGCATGATGAAACTTCG) and MTF-S (TTT GGTTTACAAGACCAATG). The sequencing of polymerase chain reaction (PCR) products was performed using the PCR primers and two internal primers (CB2F: ACAACGCAACCTTAACACGATT and CB3R: GGTGGAATGGGATTTTATCTG) by Macrogen (www.macrogen.com). All sequences used in this study are deposited in GenBank (accession nos. EU557098–EU557193). The Bayesian method (MRBAYES v. 3.1) was used to reconstruct the tree (Huelsenbeck & Ronquist 2001) based on an optimized model of sequence evolution (HYK+I+gamma), determined for the specific dataset by the Bayesian information criterion in MRAIC v. 1.4.3 (Nylander 2004). Two independent runs of five simultaneous Markov chains (one cold and four heated) were run for 10 million generations, sampling the chains every 1000 generations. The first 5 million generations were discarded as the burn-in. Convergence was checked by plotting the parameters against generations, and using the diagnostic tools available in MRBAYES v. 3.1. A 50% majority-rule consensus tree

('Bayesian' tree) was then constructed and node support was considered significant when more than 95% of the sampled trees recovered a particular clade (Huelsenbeck & Ronquist 2001).

This phylogeographic analysis of the *A. roquet/ extremus* complex gives a well-supported tree with four primary clades in Martinique, as shown in figure 4. The spatial distribution of these clades can be tested against these geological regions using matrix correspondence, which shows an extremely close association between the four clades and four out of the five precursor islands (Thorpe & Stenson 2003). This suggests that all the precursors, except Caravelle in the east, have populations surviving from a much earlier time when they were separate islands. Once again, although interpretations of times of island origin may vary, the molecular clock interpretations are compatible with island times, indicating that these separate Martinique precursor islands were occupied from around the time of origin of the young arc in this area, *ca* 6–8 Myr BP (figure 2*b*; Thorpe & Stenson 2003; Thorpe *et al.* 2005*b*). The chronology of the uplift of the central area is less easy to time geologically, but molecular clock estimates of divergence can be made using penalized likelihood (Sanderson 2002, 2003) using the four internal geological calibration points in Thorpe *et al.* (2005*b*). This suggests a time of 1.5 Myr BP for the central lineage node (figure 4, node 1) and *ca* 1.1 Myr BP for subsequent nodes encompassing the widest geographically distributed sublineages within the central (figure 4, nodes 2 and 3). Hence, it appears that the uplift required to join all the precursors into the single island of Martinique may have been as recent as 1.5 Myr BP or less. The combination of phylogeography and geology therefore suggests that these island populations have diverged in allopatry for as long as most allopatric island species in this series and other Lesser Antillean anole series (Thorpe *et al.* 2008), and have made secondary contact relatively recently. Indeed, the Barbadian species (*Anolis extremis*) is in fact paraphyletic to *A. roquet*, being a sister lineage to the central Martinique lineage and nested within the remaining Martinique lineages (figure 4). Hence, the Martinique lineages are de facto phylogenetically deeper than this nominal species.

Taken overall, the Martinique phylogeography, timing and interspecific phylogeny indicate that the Martinique precursor lineages are the equivalent of the current nominal allopatric island species, and their interaction on secondary contact is a sound test of the contribution of allopatry to speciation in this group. Since Martinique has strong environmental zonation, to which the anoles are known to adapt, a perspective can be gained by comparing the extent of genetic interaction associated with these ecotones with that associated with the secondary contact of the previously allopatric populations.

(b) *Transects*
The relative impact of allopatry and ecotones in this complex is tested by taking transects across the ecotones and secondary contact zones and investigating the genetic interaction across these zones. Here, we exemplify the approach using just one transect that
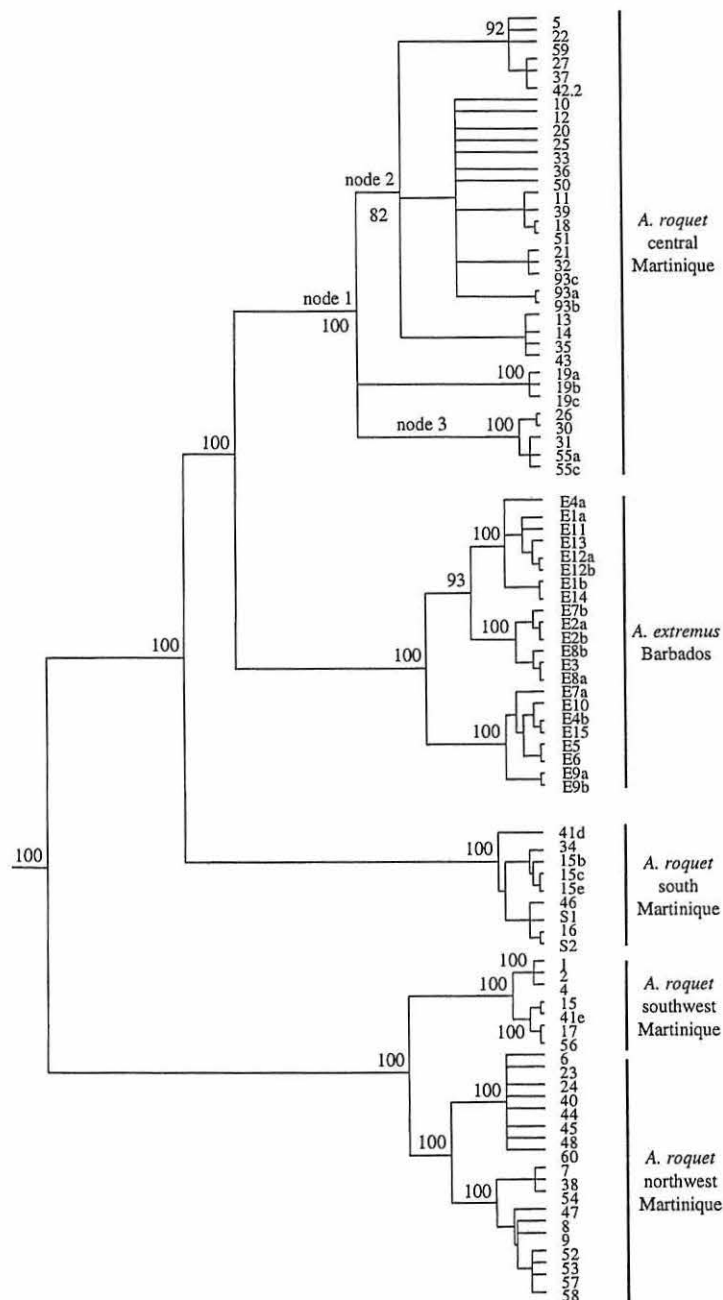
Figure 4. Phylogeny of *Anolis roquet* and associated species. This Bayesian tree shows the Barbadian species, *A. extremus*, is the sister lineage to central Martinique and is nested within the phylogenetically deeper divisions of *A. roquet* from the precursor islands, which are now in secondary contact. The terminal node numbers are the locality codes for Martinique in Thorpe & Stenson (2003) and Barbados in Thorpe *et al.* (2005*b*), which are followed by alphabetic codes for individual replicates, with E denoting *A. extremus*, and additional localities 93, S1 and S2 (located at UTM zone 20 coordinates E-720425/N-1630450, E-733300/N-1599225 and E-732157/N-1594585, respectively).

goes across both ecotone and secondary contact zone. The transect goes from the xeric woodland of the north Caribbean coast to the central montane rainforest. In doing so, it also crosses from the northwest geological region to the central geological region, hence crossing both ecotones and secondary contact zones. Figure 3

illustrates the seven localities on this transect in relation to the ecotone and the position of secondary contact suggested by geological evidence. At each locality, 48 tail-tip biopsies were sampled for molecular analysis, while quantitative traits and dewlap hue were recorded from 10 adult males.

(i) *Lineages*

The lineages were first investigated using the complete cytochrome *b* sequence from the mtDNA. PCR-restriction fragment length polymorphism analyses were then designed to efficiently assign numerous individuals to either the northwest or central clade. The same fragment was digested after amplification using the restriction enzyme SspI (New England Biolabs) during 3 hours at 37°C. The digested products were run on a 2% agarose gel containing ethidium bromide. PCR products from the central clade are uncut by this enzyme while those from the north-western clade are cut at position 128, allowing an unambiguous assignment of the individuals to these two clades.

(ii) *Selection regimes*

Both physical and biotic conditions vary dramatically along the transect. The xeric woodland gives way abruptly to montane rainforest through a belt of transitional woodland (Lassere 1977; figure 3). In the xeric woodland of the Caribbean coast, there may be as little as just over 1000 mm per annum of seasonal rain, while in the higher altitude rainforest there may be in excess of 5000 mm per annum (or even more than 8000 mm) of less seasonal rain (Meteo France; Lassere 1977). The temperature (lower in the rainforest) also changes along the transect in concert with these changes in the extent and timing of precipitation and vegetation. Normalized principal component analysis was used to generalize these environmental variables (vegetation type, altitude, seasonality of precipitation, annual precipitation and temperature), with the latter three variables obtained from Worldclim (http://www.worldclim.org/bioclim.htm).

Here, as elsewhere in the Lesser Antilles, the quantitative traits of these anoles track these selection regimes. Parallel variation within Martinique shows that xeric forms have zebra striping whereas montane rainforest forms have intense green hue marbled with black and non-UV white spots, and a variety of scalation, body dimension and other characters show a similar pattern of variation (Thorpe 2005). A multivariate suite of quantitative traits is selected to represent the phenotypic change along the transect in response to the changing selection regime. There are seven body dimensions adjusted against snout–vent length by analysis of covariance (jaw length, head length, head depth, head width, upper leg length, lower leg length and dewlap length), the percentage red, green and blue hue on the posterior trunk assessed in Adobe Photoshop, together with seven scale and pattern characters (post-mental scales, scales between supra-orbitals, number of dorsal chevrons, chevron intensity, occipital 'A' mark, black dorsal reticulation and white spots) that could result in a heteroscedastic within-group covariance matrix. These heteroscedastic characters were

normalized and then subjected to a principal component analysis. The subsequent component scores, together with the body dimensions and hues, were subjected to a canonical variance analysis, where each transect locality is a group. The individual scores (normalized so that the pooled within-group standard deviation is unity) are plotted against the transect.

A large dewlap is present in males. In solitary anoles it is largely used for conspecific (male–male and male–female) signalling. The hue of both the anterior and posterior dewlap, from near UV to the red spectrum, was recorded as the diffuse reflectance from the surface as a percentage of a WS-2 white standard, using an AvaSpec-2048 spectrometer, with an AvaLight-XE xenon pulsed lights source (Avantes, The Netherlands), with a 200 µ receptor fibre, held at 45° to the surface by a purpose-made matt-black attachment. On each individual, at least three recordings were taken per dewlap region.

Rather than simply subjecting the very large number of autocorrelated reflectances at a given specific wavelength to a principal component analysis, the matrix-algebraic procedure in Thorpe (2002) is followed. This gives several independent wavelength segments (colours) as unit characters that can be compared across large samples of individuals at numerous localities, and is consequently suitable for this comparative population-level study. The application of this process to the Martinique (Thorpe & Stenson 2003) and related (Thorpe 2002) anoles, using both large samples and multiple body regions, yields robust results. For this study, we follow the results of Thorpe & Stenson (2003), with the exception that the near UV is divided into two segments: UV 330–380 nm; UV/violet 380–430 nm; blue 430–490 nm; green 520–590 nm; yellow/orange 590–640 nm; and red 640–710 nm. These 'colour' characters from the anterior and posterior dewlap (12 in all) were subjected to a canonical variate analysis with each transect locality as a group. As with the quantitative traits, the individual scores (with the pooled within-group standard deviation as unity) are plotted against the transect.

(iii) *Genetic structure: nDNA*

Samples were genotyped at nine microsatellite loci (AAE-P2F9, ABO-P4A9, AEX-P1H11, ALU-MS06, ARO-035, ARO-062, ARO-065, ARO-120, ARO-HJ2; Ogden *et al.* 2002; Gow *et al.* 2006) in a single multiplex using a Qiagen Multiplex PCR kit following the manufacturer's recommendations except that the annealing temperature was 55°C. PCR products were then analysed on an ABI 3130xl genetic analyser and the genotypes scored using GENEMAPPER v. 4.0 (Applied Biosystems). There were no instances of linkage disequilibrium (Gow *et al.* 2006). The genetic structure along the transect was studied using Bayesian clustering as in the program STRUCTURE v. 2.1 (Pritchard *et al.* 2000). We defined the number of populations (K) from 1 to 9, and 10 independent runs were performed for each value of K using the admixture model, a burn-in of 100 000 steps followed by 400 000 post-burn-in iterations. We used the method described by Evanno *et al.* (2005) to determine the optimal number
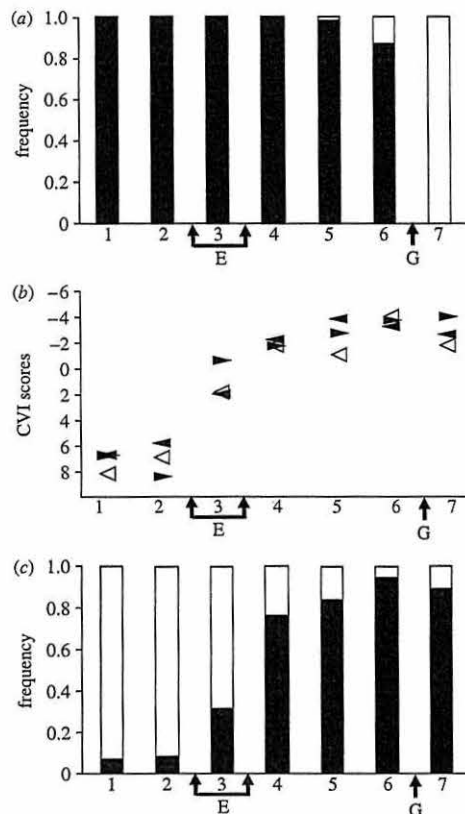
Figure 5. Transects showing (*a*) lineages, (*b*) selection regimes and (*c*) genotype structure. The transect localities (mapped in figure 3) are arranged in geographical order on the horizontal axis, with E representing the position of the ecotone (the switch between xeric coastal woodland and montane rainforest) and G the position of secondary contact between the northwest (localities 1–6) and central (7) geological regions. (*a*) The frequency (48 individuals per locality) of the northwest (black) and central (white) mtDNA lineages. (*b*) Mean first canonical variate (CVI) scores for adult males based on their quantitative traits (right-pointing filled triangles) and dewlap hue (left-pointing filled triangles), and the first principal coordinate score of environmental variables (open triangles). The CV scores are in units of 2 within group standard deviations. (*c*) The frequency of individuals (48 individuals per locality) assigned to the two Bayesian clusters, 'xeric' (white) and 'montane rainforest' (black), based on variation in nine unlinked microsatellites.

of populations. Finally, we studied the partitioning of the genetic variance within and among predefined groups with an analysis of molecular variance (AMOVA) using the program ARLEQUIN v. 3.11 (Excoffier *et al.* 2005). We carried out two different AMOVAs: first, grouping populations into the two geological regions and, second, grouping populations by habitat type, i.e. xeric woodland (localities 1 and 2), transitional forest (locality 3) and montane rainforest (localities 4–7). This classification is supported by both the vegetation type and the generalized multivariate environmental analysis.
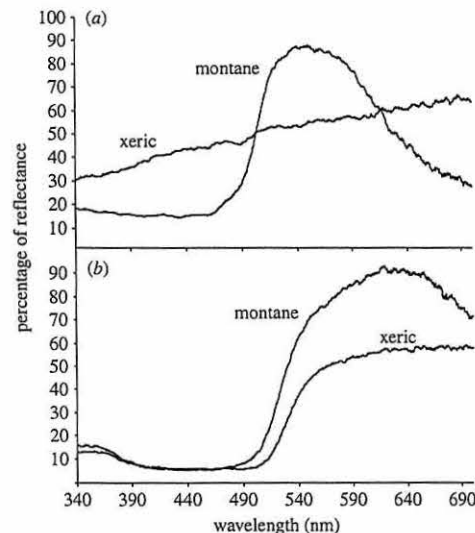
Figure 6. Dewlap hue. Examples of spectrophotometrically measured percentage reflectance across the near UV to red spectrum (in nm). (*a*) The anterior dewlap of a xeric woodland male is a dull greyish (including near UV) hue, which contrasts to the bright focal green hue of the montane rainforest male. (*b*) The posterior dewlap of the rainforest male is brighter, more focal yellow, than that of the xeric woodland male.

4. RESULTS AND CONCLUSIONS

The island-wide study of lineages on Martinique, although based on a single, or a few, specimens per locality, showed a close association between the lineages and precursor islands. The transect study, which is based on much greater sample sizes (48 specimens per locality), confirms this. The transect shows that there is remarkably little introgression of the mtDNA lineages into the area occupied by the adjacent lineage (figure 5*a*). At locality 7, there are 100% central clade haplotypes, while at the adjacent locality there are 88% northwestern clade haplotypes.

All three character sets providing insights into the selection regime (environmental variables, quantitative traits and dewlap hue) change in concert along the zone (figure 5*b*), with both the quantitative traits and dewlap hue being strongly correlated with the generalized environmental component ($r = 0.96$ and 0.97, $p < 0.001$, respectively). All three change, as expected, at the ecotone between xeric woodland and montane rainforest. There is a very pronounced change in the quantitative traits across the transect, there being approximately 15 within-locality standard deviations between the extreme montane and xeric forms (figure 5*b*). The dewlap hue also changes dramatically across the ecotone, there being 10 within-locality standard deviations between the xeric and montane extremes (figure 5*b*). The anterior dewlap is light grey, including near-UV reflectance, in xeric forms, but bright focal green without notable UV reflectance in montane forms, while the posterior dewlap is brighter, more focal yellow, in the rainforest forms compared with xeric forms (figure 6).

If isolation in allopatry results in reproductively isolated species, then there should be little or no gene exchange between the genomes of the lineages from the precursor islands. If this is the case, then the nuclear DNA, as reflected in the microsatellite variation, should be distinct, and the Bayesian assignment should assign individuals into two groups reflecting their precursor island origin. This clearly does not occur (figure 5c). The distribution of the mtDNA lineages is distinct and remarkably faithfully segregated between the precursor island regions, yet the Bayesian assignment based on microsatellite variation shows no indication at all that the individuals either side of the secondary contact zone are assigned to different groups. The Bayesian analysis recognized two clusters, which we can refer to as the xeric and montane nuclear genotypes. Localities 6 and 7, either side of the secondary contact zone, both have predominantly (more than 90%) the same (montane) nuclear genotype group, suggesting that there is no discernable barrier to genetic exchange across this region of the zone. There is no significant correlation ($p \geq 0.05$) between the frequency of nuclear genotype group and the frequency of the mtDNA haplotype group along the transect. The AMOVA results confirm this, there being no significant variance associated with grouping by lineage ($p = 0.56$). By notable contrast, the nuclear genotype changes at the ecotone from a predominantly xeric nuclear genotype at localities 1 and 2 (more than 92%) to the predominantly montane nuclear genotype at localities 4–7 (76–95%), with a strong correlation with the selection regimes (generalized environmental variables $r = 0.96$, $p < 0.001$; quantitative traits $r = 0.93$, $p < 0.005$; dewlap hue $r = 0.98$, $p < 0.001$). Once again, the AMOVA results confirm this with a significant variance associated with ecological grouping (3.58%, $p = 0.007$ between ecological groups compared with 1.07% between localities within ecological groups).

The broad biogeographic pattern for Lesser Antillean anoles suggests the importance of allopatry in speciation, but this population-level study clearly questions this hypothesis. The lineages on the Martinique precursor island diverged in allopatry a long time ago, and are certainly phylogenetically deeper than some named species in the series and the *bimaculatus* series to the north (Thorpe *et al.* 2008), but there is no indication of any reduced genetic exchange between them in nDNA genes, let alone full reproductive isolation. This suggests that some of the nominal species in this series are not reproductively isolated and, of more general importance, allopatry, even for long periods, may not be sufficient to result in speciation (*sensu* even partial reproductive isolation).

By contrast, there is notable evidence of reduced nuclear genetic exchange between the anoles from the adjacent habitat types (xeric versus montane rainforest), without any physical barrier to gene flow. This may involve both natural and sexual selection. There is overwhelming evidence of the link between quantitative traits and natural selection regimes in the anoles from mountainous Lesser Antillean islands, and the quantitative traits and pattern of restricted gene exchange are strongly linked. The dewlap hue, possibly influenced by the interaction between natural and sexual selection, is also very closely linked

with the pattern of nuclear gene exchange. The mechanism for this may well involve the different light regimes in these habitat types and its impact on visual communication, i.e. the role of sensory drive in speciation (Endler 1992; Boughman 2002). This may be the case, even though the generalizations about dewlap hue and habitat type (which are not derived from Lesser Antillean anoles; Leal & Fleishman 2004; Losos in press) do not necessarily appear to pertain to these Lesser Antillean anoles. The easiest mechanism to envisage is one of assortative mating where females choose males with the 'correct' dewlap hue. However, demonstrating female choice in anoles, and other lizards, is not straightforward and male–male competition, rather than female choice, may dominate sexual selection.

The lack of introgression in the mtDNA between precursor island regions is notable compared with the situation with the nDNA microsatellite markers, and incongruence between mtDNA and nDNA patterns can be seen in other organisms (Moritz 1994; Avise 2004), including Scandinavian brown bears where Waits *et al.* (2000) remarked that nDNA genetic differentiation, as measured by microsatellite loci, is not consistent with mtDNA phylogeographic groupings. Given the difference in mutation rates, variability, recombination and effective population size, there is no expectation that the patterns of variation in space should be the same in the mtDNA and the microsatellites. These differences are why mtDNA is widely used to reveal phylogeographic relationships (Avise 2000), while hypervariable microsatellites are widely used to reveal more recently formed population genetic structure (Freeland 2005). As males generally transmit only nDNA and not mtDNA, sex-biased dispersal may be important (Waits *et al.* 2000). Anoles, like many lizards, are polygynous (Andrews & Nichols 1990; Jenssen *et al.* 2001), which predicts greater male dispersal (Dobson 1982; Pusey 1987; Perrin & Mazalov 2000) that in turn would lead to proportionally greater nDNA gene flow. There is little direct observational data available on sex-biased dispersal in anoles, although Doughty & Sinervo (1994), Doughty *et al.* (1994) and Massot *et al.* (2003) gave some evidence of male-biased dispersal in other iguanids. However, there is genetic evidence of sex-biased dispersal in *Anolis oculatus* on Dominica (Stenson *et al.* 2002) and *A. roquet* on Martinique (R. S. Thorpe, Y. Surget-Groba & H. Johansson 2008, unpublished data) from microsatellite studies. The situation is further complicated by sperm retention and multiple insemination in *Anolis* (Calsbeek *et al.* 2007). Recent common garden experiments on anoles (Eales *et al.* 2008) suggest that a female may carry up to six viable eggs (excluding those lost in transit) that are laid at two-week intervals. Hence, a dispersing gravid female may carry up to seven different diploid nuclear genomes (one from the female and six from the males), but only the one type of mtDNA haplotype.

Taken together, these factors suggest a greater capacity for nDNA gene flow, and may explain why the lack of reproductive isolation has allowed nDNA introgression while retaining a strong mtDNA phylogeographic signature. An alternative explanation is that

the nDNA markers are under selection, or are linked to loci under selection. However, on balance, we consider this as improbable, because all the nDNA markers are unlinked. So they would not only have to be linked to different loci that are under selection, but the direction of the selection on these independent loci would have to be congruent with one another and also with the habitat type.

The anoles from different habitat types have reduced genetic exchange rather than no (or almost no) genetic exchange, so we are not suggesting that these are full species generated by ecological speciation. However, this evidence of reduced genetic exchange among habitat types may well have general implications for the role of ecology in speciation in other sexually reproducing animals. In the Greater Antillean anoles, most speciation has occurred within islands (Losos in press) and, in the light of the evidence presented here, models emphasizing the role of ecology over allopatry should be given serious consideration for these and other sexually reproducing animals. This is compatible with the growing literature highlighting the role of ecology in speciation across a range of organisms (Smith *et al.* 1997; Orr & Smith 1998; Schluter 2000; Rundle & Nosil 2005; Gross 2006; Hendry *et al.* 2007; Butlin *et al.* in press; Lowry *et al.* 2008).

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Microsatellite data show evidence for male-biased dispersal in the Caribbean lizard *Anolis roquet*

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Abstract

Dispersal is a key component of an organism's life history and differences in dispersal between sexes appear to be widespread in vertebrates. However, most predictions of sex-biased dispersal have been based on observations of social structure in birds and mammals and more data are needed on other taxa to test whether these predictions apply in other organisms. Caribbean anole lizards are important model organisms in various biological disciplines, including evolutionary biology. However, very little is known about their dispersal strategies despite the importance of dispersal for population structure and dynamics. Here we use nine microsatellite markers to assess signatures of sex-biased dispersal on two spatial sampling scales in *Anolis roquet*, an anole endemic to the island of Martinique. Significantly higher gene diversity (H_S) and lower mean assignment value (mAIC) was found in males on the larger spatial sampling scale. Significant heterozygote deficit (F_{IS}), lower population differentiation (F_{ST}), mAIC and variance of assignment index (vAIC) was found in males on the smaller spatial scale. The observation of male biased dispersal conform with expectations based on the polygynous mating system of *Anolis roquet*, and contributes to an explanation of the contrasting patterns of genetic structure between maternal and biparental markers that have been reported previously in this, and other anoline, species.

Keywords: *Anolis roquet*, microsatellite, sex-biased dispersal

Introduction

Dispersal is a key component of an organism's life history, affecting both the evolution and persistence of a species (Clobert *et al.* 2001). Dispersal influences the rate of differentiation between subpopulations and the degree to which populations function as independent demographic units (Palo *et al.* 2004). Hence, understanding the dispersal pattern of an organism is a fundamental requirement for accurate inferences about population structure and dynamics. In sexual species, dispersal often occurs predominantly in one of the sexes (sex-biased dispersal). There are three hypotheses that are commonly invoked to explain the disparity in dispersal between the sexes: (i) competition among related females for resources [local resource competition (Greenwood 1980)]; (ii) competition between related males for mates [local mate competition (Dobson 1982; Perrin & Mazalov 2000)]; and (iii) avoidance of inbreeding (Pusey 1987). These hypotheses are not mutually

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exclusive and share one common facet in that they predict male-biased dispersal in taxa with polygynous mating systems. Conversely, for monogamous species, only local resource competition predicts a bias in dispersal, and this bias is in favour of dispersal among females (Greenwood 1980). These predictions are broadly supported by empirical evidence from mammals and birds; in mammals (often polygynous), males normally disperse further from their natal area, whereas in birds (often monogamous), female-biased dispersal predominates (Greenwood 1980; Handley & Perrin 2007). Nevertheless, there are examples of species of mammals and birds that do not conform to the general patterns (Clarke *et al.* 1997; Gibbs *et al.* 2000; Dallimer *et al.* 2002; Moller & Beheregaray 2004; Williams & Rabenold 2005; Broquet *et al.* 2006; Handley & Perrin 2007), suggesting that mating system hypotheses cannot be applied universally. Moreover, recent studies have suggested that kin selection and sociality may play an important part in the evolution of sex-biased dispersal (Devillard *et al.* 2004).

Studies on species in other taxa, for example salmonids (Hutchings & Gerber 2002; Bekkevold *et al.* 2004; Fraser *et al.* 2004; Palstra *et al.* 2007), cichlids (Knight *et al.* 1999; Taylor

et al. 2003) and frogs (Austin *et al.* 2003; Lampert *et al.* 2003; Palo *et al.* 2004) are relatively few and the patterns of sex-biased dispersal are equivocal. Likewise, there are relatively few published studies that examine patterns of sex-biased dispersal in lizards. Monogamy in lizards is relatively rare; however, in the few known examples microsatellite data has revealed that slight female bias in dispersal appears to be the norm (Bull 2000; Gardner *et al.* 2001; Stow *et al.* 2001; Chapple & Keogh 2005) in line with predictions from mating system and local resource competition. In lizards with polygynous mating systems, patterns of sex-biased dispersal vary. In the common lizard (*Lacerta vivipara*) a mark–recapture study shows slight male-biased dispersal, where dispersal rates are dependent on female density and kinship (Lena *et al.* 1998), while microsatellite data showed an indication of male-biased dispersal in *Anolis oculatus* (Stenson *et al.* 2002) and, from mark–recapture data, in *Sceloporus occidentalis* (Massot *et al.* 2003). Mark–recapture studies in *Uta stansburiana* showed that males disperse further than females in some years, but not in others (Doughty & Sinervo 1994; Doughty *et al.* 1994). However, mark–recapture data from *Lacerta agilis* suggest that juvenile females disperse further than males (Olsson *et al.* 1996). Both natal and breeding dispersal are also higher in females of the alpine lizard, *Niveoscincus microlepitodus*, according to mark–recapture data (Olsson & Shine 2003). Hence, with only a limited number of studies in lizards, no strong general pattern of sex-biased dispersal seems to emerge. Further investigation of sex-biased dispersal in different taxonomic groups is necessary in order to make cross-taxa comparisons with the patterns observed in birds and mammals, and to develop a more general framework for the evolution of sex-biased dispersal.

Anoles have long been recognised as important model organisms in various biological disciplines (Roughgarden 1985; Losos 2004; Lovern *et al.* 2004; Schluter 2000; Thorpe *et al.* 2004), but there have been only limited field (Andrews & Rand 1983) and genetic (Stenson *et al.* 2002) studies of dispersal in this genus. Here we investigate genetic evidence for sex-biased dispersal in *Anolis roquet*, one of the nine species in the *roquet* series of anole lizards that inhabit the Southern Lesser Antilles. *A. roquet* is an arboreal insectivorous lizard that is endemic to Martinique, and which is found in high densities across most of the island. A recent phylogenetic study on *A. roquet* revealed four very distinct mitochondrial DNA (mtDNA) lineages that were found to be closely associated with geographical regions (Thorpe & Stenson 2003). These regions correspond to peripheral precursor islands that were joined by the uplifting of a central region (Andreieff *et al.* 1976; Bouysse *et al.* 1983; Maury *et al.* 1990; Sigurdsson & Carey 1991). The phylogeographic pattern suggests that young precursor islands were colonized by anoles, and individual *A. roquet* lineages evolved in geographical isolation (allopatry) until the joining of the precursor islands (Thorpe & Stenson 2003). Following secondary contact, a distinct geographical pattern of mtDNA lineage distribution persists and lineage transitions occur over very short geographical distances (Ogden & Thorpe 2002; Thorpe *et al.* 2008). In contrast, nuclear microsatellite data has revealed a pattern of high nuclear gene flow across these secondary contact zones (Ogden & Thorpe 2002; Thorpe *et al.* 2008). This type of pattern is commonly seen in organisms with male-biased dispersal (Taberlet & Bouvet 1994; Gibbs *et al.* 2000; Waits *et al.* 2000; Castella *et al.* 2001; Petit *et al.* 2001), a hypothesis that has not been tested in *A. roquet*. Given that anoles generally have polygynous mating systems (Jenssen *et al.* 2001) predicting male-biased dispersal, it is important to test hypotheses of sex-biased dispersal as a contributing factor to the contrasting patterns between mtDNA and nuclear DNA in this and other anoline species (Stenson *et al.* 2002).

Direct estimations of dispersal through mark–recapture studies are time-consuming and expensive due to the extensive fieldwork required (Berry *et al.* 2004). Furthermore, *A. roquet* is a small, arboreal, often cryptic animal that inhabits a complex tropical habitat. Hence, it would be particularly difficult to survey dispersing juveniles efficiently. Moreover, in anoles there is a high turnover, particularly among juveniles (Andrews & Rand 1983), which would require a very high number of individuals to be marked in order to reliably estimate dispersal. An indirect approach based on microsatellite frequencies offers an attractive alternative to mark–recapture studies (Goudet *et al.* 2002; Prugnolle & de Meeus 2002; Berry *et al.* 2004; Handley & Perrin 2007). Sex-biased dispersal affects genetic structure between and within populations, which can be detected by calculating indices from polymorphic genetic data. Results from these methods based on genetic data have been favourably compared with mark–recapture studies (Favre *et al.* 1997).

Materials and methods

Anolis roquet is a sexually dimorphic lizard; mature males are larger than females and show distinctive markings. Both males and females have dewlaps, however, the dewlap on males is larger and more brightly coloured (Lazell 1972), hence discrimination between adults from each sex is straightforward. Only adult lizards were used for this study.

We sampled a total of 17 localities on two spatial scales. Ten island-wide localities (distance between localities ranged between 4–43.6 km, the mean being 17.12 km) were sampled between March and May 2005–07. Localities were chosen to incorporate at least one locality from each mtDNA lineage and cover as many habitat types as possible. Seven further localities situated along a 4-km transect, all in the same habitat type (Fig. 1), were sampled between April and May 2007. Distances between these latter localities are likely to be a more realistic scale for juvenile dispersal. Each
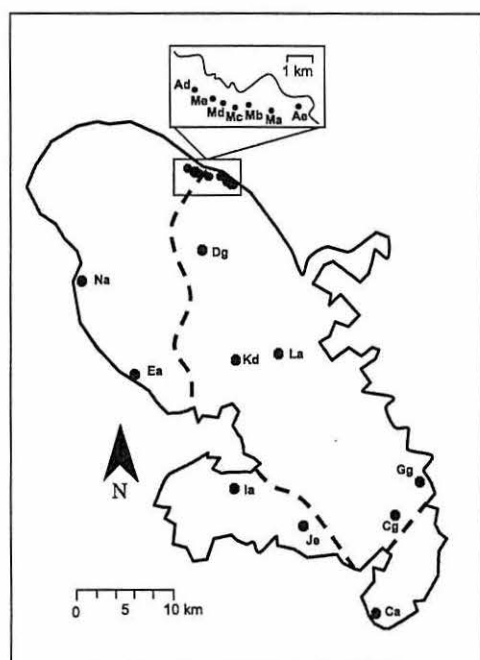
Fig. 1 Map of Martinique, showing the island-wide sampling localities and the transect at the smaller sampling scale (inset). Broken line shows the phylogenetic lineage boundaries for the four main mtDNA lineages of *Anolis roquet* on Martinique.

locality (at both sampling scales) was sampled over no more than two days in the same year. Only data from locations yielding at least 28 adult individuals and approximately equal numbers of females and males were included in this study.

Autotomised tail tips were collected for DNA analysis and stored in 100% ethanol. Genomic DNA was extracted by the Chelex method described by Estoup *et al.* (1996). Individuals were typed at nine microsatellite markers (Ogden *et al.* 2002; Gow *et al.* 2006; Johansson *et al.* 2008). Loci were amplified in a multiplex polymerase chain reaction (PCR) (primer concentrations: 0.05 μM for ARO-035, ARO-062, ARO-065 and ARO-HJ2, 0.1 μm, for ARO-120 and 0.2 μm for ABO-P4A9, AAE-P2F9 and ALU-MS06) using QIAGEN Multiplex PCR kit following the manufacturers instructions, with the exception of an annealing temperature of 55 °C. The amplified products were then analysed on an ABI 3130xl genetic analyser with the internal size standard 600-LIZ, and genotypes scored using GENEMAPPER version 4.0 (Applied Biosystems).

From the ten localities sampled island-wide, a total of 372 individuals were genotyped, ranging from 28–48 individuals per population (mean 39.5), of which 184 were female and 188 were male. The proportions of sexes genotyped from each locality can be found in Table 1. From the transect, a total of 281 individuals were genotyped, with 36–44 individuals per locality (mean 40.1), totalling 140 females and 141 males (Table 1).

Genetic data from the two sets of localities were analysed separately. The software ARLEQUIN version 3.01 (Schneider *et al.* 2000: http://lgb.unige.ch/arlequin/) was used for exact testing of Hardy–Weinberg equilibrium (Guo & Thompson 1992) and calculation of linkage disequilibrium (Slatkin & Excoffier 1996) for each population and locus. Departure from Hardy–Weinberg equilibrium was considered with, and without, Bonferroni correction. For all calculations of *F*-statistics we used Weir & Cockerham's (1984) unbiased estimators. To test for overall genetic differentiation in the two samples and for pairwise differentiation between individual populations, we used FSTAT version 2.9.3 (Goudet 1995: http://www2.unil.ch/popgen/softwares/fstat.htm) to calculate global and pairwise F_{ST}, respectively.

To test for sex-biased instantaneous dispersal we calculated the gene diversity (H_S), F_{IS}, F_{ST}, mean assignment index (mAIC) and variance of the assignment index (vAIC) separately for each sex. Statistical significance for these indices was determined by 10 000 randomizations as implemented in FSTAT version 2.9.3. A bias in dispersal between the sexes should be reflected in statistically significant dissimilarity in the estimated parameters. The higher-dispersing sex should have a higher F_{IS}: in the dispersing sex individuals sampled from one single patch will be a combination of residents and immigrants, hence a heterozygote deficit is expected due to the Wahlund effect (Goudet *et al.* 2002). More similar allele frequencies are expected between sampling sites in the dispersing sex and less differentiation between populations. Thus F_{ST} is expected to be higher in the philopatric sex and within-site gene diversity (H_S) is expected to be lower (Goudet *et al.* 2002). The measure of F_{ST} (i.e. among-population differentiation) on the island-wide sampling scale is unlikely to be informative with respect to actual dispersal rates as it is unlikely that populations exchange migrants over these distances. The probability of a genotype originating in the population from which the genotyped individual was sampled can be calculated as an assignment index, from which the sample mean (mAIC) can be found (Paetkau *et al.* 1995). A relatively higher frequency of rarer genotypes is expected in populations of the dispersing sex and this is indicated by a negative assignment index (Paetkau *et al.* 1995; Prugnolle & De Meeus 2002). Finally, the vAIC can be estimated from mAIC, where variance is expected to be larger for the sex that disperses (Favre *et al.* 1997). In the case of microsatellite data (or other biparental markers), these methods detect only short-term dispersal,

Locality	UTM Easting	UTM Northing	$N_{(T)}$	$N_{(F)}$	$N_{(M)}$
Island-wide localities					
Ca	728245	1593645	28	15	13
Cg	730021	1604099	38	19	19
Dg	709480	1632216	35	17	18
Ea	702159	1618280	48	25	23
Gg	732909	1607713	32	14	18
Ia	712794	1606954	41	22	19
Je	720504	1603147	34	17	17
Kd	713197	1621052	43	19	24
La	717409	1621378	38	19	19
Na	696124	1629433	35	17	18
Transect localities					
Ad	708108	1640748	40	20	20
Ae	711603	1640234	36	18	18
Ma	710657	1640090	40	20	20
Mb	709933	1640253	44	22	22
Mc	709442	1640153	38	19	19
Md	709089	1640332	40	20	20
Me	708722	1640418	43	22	21

Table 1 Locality names, GPS position (UTM Easting and UTM Northing), total number of genotyped samples ($N_{(T)}$), number of females ($N_{(F)}$) and number of males ($N_{(M)}$) for each locality

Table 2 Deviations from Hardy–Weinberg expectations (F_{IS}), F_{ST}, gene diversity (H_S) mean assignment index (mAIC) and variance of mean assignment index (vAIC) for the ten island-wide localities for females (F) and males (M). P-values are from two-tailed tests where; ** = significant at $P < 0.01$

	F_{IS}	F_{ST}	H_S	mAIC	vAIC
F	0.012	0.062	0.756	0.502	9.719
M	0.022	0.057	0.778	−0.491	11.533
P-value	0.597	0.404	0.005**	0.003**	0.406

Table 3 Deviations from Hardy–Weinberg expectations (F_{IS}), F_{ST}, gene diversity (H_S) mean assignment index (mAIC) and variance of mean assignment index (vAIC) for the seven transect localities for females (F) and males (M). P-values are from two-tailed tests where; ** = significant at $P < 0.01$, * = significant at $P < 0.05$

	F_{IS}	F_{ST}	H_S	mAIC	vAIC
F	−0.009	0.014	0.791	0.345	9.211
M	0.044	0.006	0.795	−0.347	13.574
P-value	0.003**	0.025*	0.301	0.047*	0.014*

since this signal disappears after the dispersing individuals mate, due to the Mendelian segregation of biparental markers (Goudet *et al.* 2002).

Results

The mean number of alleles scored per population (averaged over all loci) from the ten island-wide localities sampled was 10.4. There was no evidence of consistent departures from Hardy–Weinberg equilibrium or of linkage disequilibrium in the samples (Table S1). The global F_{ST} was estimated at 0.059, with pairwise F_{ST} values ranging from 0.0157 to 0.1227, suggesting moderate levels of genetic differentiation on the sampled spatial scale. Males display significantly higher gene diversity, H_S (males = 0.778, females = 0.756, $P = 0.005$) and significantly lower mAIC values (males = −0.491, females = 0.502, $P = 0.003$). The remaining indices (F_{IS}, F_{ST} and vAIC) were compatible with male-biased dispersal, but did not reveal statistically significant differences (Table 2).

The mean number of alleles scored per population on the transect was 10.62. No consistent departures from Hardy–Weinberg equilibrium and no linkage disequilibrium were detected. The global F_{ST} was estimated at 0.011, with pairwise F_{ST} values ranging from 0.0024–0.0213, suggesting low levels of genetic differentiation. Four tests of sex-biased dispersal were found to be significant on this sampling scale. Females had a negative F_{IS} (−0.009) compared to the positive value for males (0.044), showing a highly significant difference ($P = 0.003$). The variance of assignment index also showed a significant difference ($P = 0.014$), with males having a higher index (13.574) compared to females (9.211). Females also showed significantly higher differentiation compared to males (female $F_{ST} = 0.014$, male $F_{ST} = 0.006$, $P = 0.025$). Furthermore, males and females differed significantly in mAIC, with males returning a negative value of −0.347, while females showed a positive value of 0.345 ($P = 0.047$). The difference in gene diversity was not significant, although the level in males was on average higher (Table 3).

Discussion

We included island-wide sampling (covering lineages and habitats) so that, in spite of limitations, we could generalize some of our findings to the entire species. The distances between the island-wide populations are well beyond the distance juvenile anoles are expected to disperse, on account of their small size. Furthermore, the ten island-wide localities were sampled during different years. These two factors mean that among-population indices (pairwise F_{ST}'s) cannot be used to infer sex-biased dispersal at this scale (Goudet *et al.* 2002), but the within-population indices are informative as they are not affected by the sampling protocol. The transect localities were sampled in the same year and in the same habitat, and given the short distance between the localities, pairwise comparisons are expected to be reliable.

All of the indices estimated, on both spatial sampling scales, suggest that males have a higher dispersal rate than females in *Anolis roquet*. Highly significant differences between males and females were observed in the estimates of mean assignment index and gene diversity on the island-wise sampling scale, whereas F_{IS}, F_{ST}, mAIC and vAIC showed significant differences on the transect. The evaluation by Goudet *et al.* (2002) of the efficiency and power of these tests showed that test performance was dependent on dispersal rates, strength of bias, polymorphism of the markers and the sampling. These tests generally have a low power in detecting bias, unless sex-bias in dispersal is at least 80:20 and all populations involved are sampled exhaustively (Goudet *et al.* 2002). Within this context, the significant indices detected in this study provide strong evidence for male-biased dispersal in this species. The results for *A. roquet* are in concordance with the prediction of male-biased dispersal in polygynous species, which was first suggested by Greenwood (1980). However, not all polygynous lizards show male-biased dispersal (Olsson *et al.* 1996; Olsson & Shine 2003), and several reasons can be postulated for the results observed in *A. roquet*.

It has been shown that in cases where males compete for females (local mate competition) and females compete for resources (local resource competition), the association between male-biased dispersal and polygyny is strengthened (Perrin & Mazalov 2000). Similarly, higher levels of competition between males than between females in a polygynous system increase the likelihood of a male-bias in dispersal. This occurs in the female defence polygyny observed for sexually dimorphic members of *Anolis* (Trivers 1976; Schoener & Schoener 1980; Jenssen *et al.* 2001). Female anoles choose their territory before they reach sexual maturity and several females can hold home ranges that overlap considerably (Trivers 1976; Jenssen *et al.* 2001). Males subsequently enter female territory and dominant males defend their access to, and mate with, several females (Schoener & Schoener 1980; Jenssen *et al.* 2001). Therefore, females may

benefit from dispersing only to the extent to which they are able to obtain adequate resources for survival and reproduction (Stamps 1977), whereas males may have to disperse further to find territory that is unoccupied by superior males. In those anoles that have polygynous mating systems it appears that intrasexual aggression levels are consistently higher in males than in females (Trivers 1976; Jenssen *et al.* 2000), and this aggression has been hypothesised to cause juvenile or subadult males to leave their natal area (Trivers 1976; Schoener & Schoener 1980; Schoener & Schoener 1982).

Conversely, philopatry is typically favourable for the sex that invests highly in their offspring, given that knowledge of the territory and potential social interactions with kin may provide benefits (Greenwood 1980; Waser & Jones 1983). In anoles, as in most species of lizard, there is no post-hatchling parental investment, however, females invest more energy in reproduction (for egg production) than males (Orrell *et al.* 2004) suggesting benefits for philopatric females. As described above, there is a very strong mtDNA structure in *A. roquet*. Specific lineages are associated with the different precursor islands, even after an estimated 1.5 million years of secondary contact and in the absence of physical or ecological barriers (Ogden & Thorpe 2002; Thorpe *et al.* 2008). Such a pattern has been described in several species (Taberlet & Bouvet 1994; Gibbs *et al.* 2000; Waits *et al.* 2000; Castella *et al.* 2001; Petit *et al.* 2001) and has been explained by very high philopatry in females. A comprehensive study of this type of pattern comes from the greater mouse-eared bat *Myotis myotis* (Castella *et al.* 2001). In this species females aggregate to form nursing colonies in spring and summer, and the females have been shown to exhibit strong fidelity to their natal colonies, whereas the geographic origin of males is usually unknown. Castella *et al.* (2001) show that dispersal of effectively a single sex in *M. myotis* is sufficient to homogenize nuclear DNA structure whilst preserving mtDNA structure, when the other sex is philopatric. Similar conclusions were drawn by Bowen *et al.* (2005), in a study on loggerhead turtles. Hence the structure observed in *A. roquet* mtDNA is probably due to strong female philopatry, as observed previously in *Anolis oculatus* by Stenson *et al.* (2002).

In species with strong philopatric tendencies, sex-biased dispersal is beneficial since it decreases the risk of mating with related individuals (Pusey 1987). Furthermore, the potential cost of inbreeding associated with philopatry may also be reduced by multiple paternity in anoles. Some female anoles have been shown to be highly promiscuous, and able to store sperm for more than two months (Fox 1963; Calsbeek *et al.* 2007; Eales *et al.* 2008). Offspring of promiscuous females are genetically more diverse, thereby allowing maternal half-siblings to mate with a decreased risk of inbreeding (Calsbeek *et al.* 2007).

In conclusion, this study shows strong evidence for male-biased dispersal in a species for which direct dispersal

estimation by mark–recapture methods would be very difficult to obtain. This result contributes to an explanation of the conflicting patterns of gene flow between nuclear and mitochondrial markers observed by Thorpe *et al.* (2008). Indeed, it seems likely that pronounced female philopatry produced the strong mtDNA lineage structure observed across the island (Stenson & Thorpe 2003), while nuclear gene flow across the lineage contact zones may be predominantly maintained by means of male dispersal.

Acknowledgements

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The authors belong to the Molecular Ecology and Evolution of Reptiles Unit in Bangor. This paper is part of the team's investigation into population genetics and speciation in the *Anolis roquet* complex and forms part of Helena Johansson's research for a PhD. Yann Surget-Groba is interested in the molecular ecology of lizards, including the evolution of viviparity. Roger S Thorpe is interested in natural selection, population genetics, molecular phylogeography and speciation of island lizards.

The roles of allopatric divergence and natural selection in quantitative trait variation across a secondary contact zone in the lizard *Anolis roquet*

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Abstract

Populations of the Caribbean lizard, *Anolis roquet*, are thought to have experienced long periods of allopatry before recent secondary contact. To elucidate the effects of past allopatry on population divergence in *A. roquet*, we surveyed parallel transects across a secondary contact zone in northeastern Martinique. We used diagnostic molecular mitochondrial DNA markers to test fine-scale association of mitochondrial DNA lineage and geological region, multivariate statistical techniques to explore quantitative trait pattern, and cline fitting techniques to model trait variation across the zone of secondary contact. We found that lineages were strongly associated with geological regions along both transects, but quantitative trait patterns were remarkably different. Patterns of morphological and mito-chondrial DNA variation were consistent with a strong barrier to gene flow on the coast, whereas there were no indications of barriers to gene flow in the transitional forest. Hence, the coastal populations behaved as would be predicted by an allopatric model of divergence in this complex, while those in the transitional forest did not, despite the close proximity of the transects and their shared geological history. Patterns of geographical variation in this species complex, together with environmental data, suggest that on balance, selection regimes on either side of the secondary contact zone in the transitional forest may be more convergent, while those either side of the secondary contact zone on the coast are more divergent. Hence, the evolutionary consequences of allopatry may be strongly influenced by local natural selection regimes.

Keywords: allopatry, *Anolis roquet*, cline, natural selection

Received 8 April 2008; revision received 25 September 2008; accepted 1 October 2008

Introduction

Hybrid zones are areas where distinct genotypes or phenotypes meet and produce hybrids (Barton & Hewitt 1985), and as such they are relevant to studies of speciation (Harrison 1991; Nurnberger *et al.* 1995; Jiggins & Mallet 2000). Hybrid zones arise from either primary or secondary contact (Barton & Hewitt 1985). In the latter case, differences built up during a period of geographical isolation are effectively put to a test of compatibility (Fitzpatrick & Shaffer 2004). On initial contact, steep and congruent clines in multiple characters may form. With time, and in the absence of strong selection, these clines can

disappear due to the homogenising effects of dispersal and recombination (Endler 1977; Barton & Hewitt 1985), and previously isolated populations may merge (Sequeira *et al.* 2005). Alternatively, the centre and width of some clines may displace and flatten (respectively) in response to varying selection pressures on different traits (Parsons *et al.* 1993; Nurnberger *et al.* 1995; Brumfield *et al.* 2001; Takami & Suzuki 2005). Finally, multiple character clines may remain steep and congruent in response to strong selection against hybrids (Dasmahapatra *et al.* 2002; Phillips *et al.* 2004), offering the possibility of continued population divergence.

Estimating the relative contribution of two types of selection, endogenous (intrinsic) and exogenous (extrinsic), is a fundamental problem in hybrid zone study (Kruuk *et al.* 1999). Endogenous selection leads to the formation of

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tension zones, which are independent of environment and predominantly maintained by reduced fitness in hybrids due to an incompatible mix of genomes (Barton & Hewitt 1985; Nurnberger *et al.* 1995; Phillips *et al.* 2004). Exogenous selection results in hybrid zones where different types may be favoured on either side of an environmental gradient, and it is the environmental gradient that ultimately determines the position of the cline (Nurnberger *et al.* 1995). In patchy environments, exogenous selection can lead to the formation of mosaic hybrid zones. In these zones, character traits show abrupt reversals and transitions in concordance with habitat distribution (Ross & Harrison 2002; Vines *et al.* 2003; Fitzpatrick & Shaffer 2004). Endogenous and exogenous selection can act together (Szymura & Barton 1986; Szymura & Barton 1991; Sites *et al.* 1995) and distinguishing the effects of one from the other can be difficult or impossible (Kruuk *et al.* 1999; Marshall & Sites 2001). The relative strengths of endogenous and exogenous selection pressures can also vary in different areas of a zone of secondary contact (Szymura & Barton 1991; Hairston *et al.* 1992; Vines *et al.* 2003) and clines can move, either as a response to change in environment (Dasmahapatra *et al.* 2002; Leache & Cole 2007), due to competition (Rohwer *et al.* 2001) or into areas of low population density (Barton & Hewitt 1985). The fate of clines in secondary contact zones is ultimately concerned with fundamental theories of speciation and species concepts. Case studies from hybrid zones can offer insight into the effects of allopatry and the importance of other forces driving population divergence upon secondary contact.

Adaptive radiations, such as the *Anolis* radiation in the Caribbean, are particularly useful for the empirical study of the processes involved in speciation. The *c.* 150 species of anoles in the Caribbean demonstrate high levels of in-situ speciation from as few as two colonisation events (Jackman *et al.* 1999). The islands involved in this adaptive radiation are grouped into two sets based on island size and geology: the Greater Antilles, which include the large islands of Cuba, Hispaniola, and Puerto Rico, and the Lesser Antilles, a chain of small islands extending from Anguilla, south towards Venezuela. The anole communities on the two sets of islands share some fundamental similarities. Specifically, there is strong evidence in both areas of natural selection acting on quantitative traits. Selection has led to both intraspecific within-island adaptation to habitat in the Lesser Antilles (Thorpe & Malhotra 1996; Malhotra & Thorpe 2000) and divergent habitat specialisation among species in the Greater Antilles (Losos 2004; Calsbeek *et al.* 2006). However, the Greater Antillean islands support multispecies anole communities with up to 55 endemic species on a single island (Losos *et al.* 2003), whereas the Lesser Antilles have only one or two native species on each island (Thorpe *et al.* 2004). In the Lesser Antilles, both the biogeographical pattern of species distribution and phylo-

genetic analyses of molecular variation suggest that anole colonisation and subsequent speciation occurred progressively from island to island (Thorpe & Stenson 2003; Thorpe *et al.* 2004, 2008), suggesting that allopatry is important for speciation in this particular set of islands.

Because the Lesser Antilles are a chain of discrete islands, their respective species of anole are geographically isolated from each other. Therefore, reproductive isolation in this group cannot usually be tested in natural conditions. However, the island of Martinique is unusual due to its geological history: it appears that it was formed into a single island from five separate precursor islands, when two precursors from the older arc (Caravelle, and St Anne peninsulas, formed during the Eocene and early Miocene) and two precursors from the younger arc (Trois-Ilets peninsula and the northwest, formed during the Miocene and Pleistocene) were joined by the uplifting of the central area between them, possibly as recently as 1.5 million years ago, or less. (Andreieff *et al.* 1976; Bouysse *et al.* 1983; Maury *et al.* 1990; Sigurdsson & Carey 1991; Thorpe *et al.* 2008). This has allowed organisms that evolved in allopatry to come into secondary contact, offering an exceptional opportunity in which to test the strength of allopatric divergence and its role in speciation, while also evaluating the importance of selective forces driving differentiation.

Martinique is inhabited by a single species of anole, *Anolis roquet*, which is an endemic, arboreal, iguanid lizard. A recent phylogenetic analysis of a 1139-bp mitochondrial DNA (mtDNA) (cytochrome *b*) fragment revealed four main monophyletic lineages (Fig. 1) (Thorpe & Stenson 2003). Across the island, these main lineages were found to be very closely associated with the geological regions described above. The age of the lineages directly corresponds to the emergence of the most recent precursor islands and regions, and it is thought that young precursor islands were colonised as they emerged (Thorpe & Stenson 2003). When volcanic activity and orogeny eventually connected the precursor islands to form present-day Martinique, lizard lineages came into secondary contact (Thorpe *et al.* 2004). Three major contact zones were identified on Martinique, where divergence between the different lineages has been estimated at between 6 and 8 million years, probably followed by less than 1.5 million years of secondary contact (Thorpe *et al.* 2008).

The mountainous younger arc Lesser Antillean islands, such as Martinique, have pronounced environmental zonation, with specific vegetation types (Beard 1948). Rainforest covers the montane interiors, and is replaced by transitional forest as altitude decreases towards the coast. Coastal habitat varies, with xeric woodland in the rain shadow of the Caribbean coast, and littoral woodland on the exposed central Atlantic coast. Furthermore, rainfall and habitat change on the southern and northern tips of the islands. As a result, in Martinique the Atlantic littoral
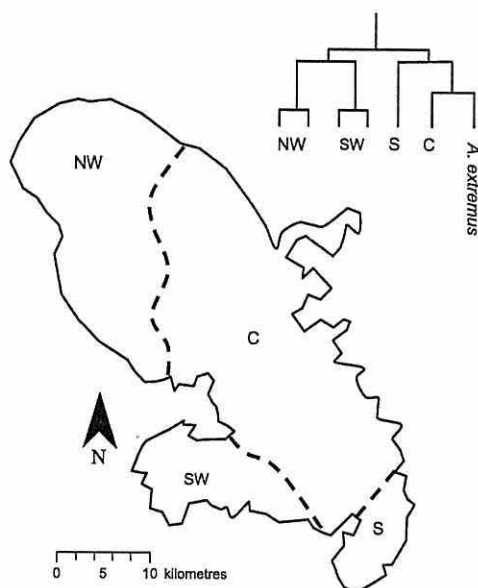
Fig. 1 Main phylogenetic relationships and lineage boundaries of *Anolis roquet* on Martinique (Thorpe & Stenson 2003; Thorpe *et al.* 2008). *Anolis extremus* on Barbados is a sister clade to the central *A. roquet* lineage (Thorpe & Stenson 2003).

woodland is replaced by xeric woodland to the south and mesic forest to the north.

The Martinique anole, like the anoles on the other Lesser Antillean islands with environmental zonation, shows marked geographical variation in hue and pattern, as well as scalation, body dimensions and size (Lazell 1972; Thorpe & Malhotra 1996; Thorpe & Stenson 2003; Thorpe *et al.* 2004, 2008). There may be specific exceptions, but overall, the geographical variation in quantitative traits (QT) of anoles on Martinique, and the other Lesser Antillean islands, is associated with this environmental zonation rather than phylogeographical lineages. This is interpreted as natural selection for current conditions and is supported by common garden experiments indicating genetic control rather than plasticity (Thorpe *et al.* 2005), large-scale field experiments on natural selection determining the intensity and targets of selection (Malhotra & Thorpe 1991; Thorpe *et al.* 2005), correlations between environmental and QT patterns (taking into account phylogeny and other factors), and parallel patterns of adaptation (Thorpe 2005). The latter is particularly notable in the Martinique anole where populations from different lineages experiencing similar environmental conditions have very similar appearance due to strong convergent selection (Thorpe 2005). An example

of this is found in montane forms which, irrespective of lineage, are an intense saturated green hue with black and non-ultraviolet (UV) white markings. Indeed, Ogden & Thorpe (2002) and Thorpe *et al.* (2008) show that where northwestern and central lineages meet in montane rainforest after prolonged allopatry, convergent selection renders them identical (in patterns of QTs) and there is no indication of reduction in gene flow across the secondary contact zone as estimated by neutral nuclear markers. In sharp contrast, divergent selection between adjacent habitats results in marked difference in QTs and a notable reduction in gene flow estimated by the same nuclear markers (Ogden & Thorpe 2002; Thorpe *et al.* 2008). Despite this general pattern, Thorpe & Stenson (2003) identified two areas where, at least superficially, quantitative trait variation seems to correlate to lineage, one in southern Martinique (Ste Anne peninsula) and the other on the north Atlantic coast.

This study examines one of the regions that may represent an exception to the general pattern. In northeastern Martinique, two of the most divergent mtDNA lineages meet (central and northwestern; 7.9% uncorrected divergence). Where these lineages meet on the coast, the Atlantic littoral woodland (Beard 1948) gives way to wetter and more seasonal climatic conditions to the north. However, inland at the transitional forest, conditions are more consistent at any given altitude, and in the montane rainforest, there is thought to be strong convergent selection for identical habitats either side of the secondary contact between these two lineages. Along the coastal strip of littoral woodland, anoles from the central lineage (C) have a brown, dull, uniform dorsum with a low UV reflective dewlap, whereas anoles from the northwestern (NW) lineage are green with black markings and a bright dewlap in the yellow/orange part of the spectrum and higher UV reflection (Fig. 2). Colour and pattern appear correlated with lineage, and 'lineage forms' are easily distinguished on the coast over the space of a few kilometres. The change is dewlap hue along the Atlantic coast of Martinique (Thorpe & Stenson 2003) may be in response to habitat change, as *A. trinitatis* on the Atlantic coast of St Vincent also has a highly UV reflective dewlap (Thorpe 2002). However, in the transitional forest, a short distance towards the interior of the island, lizards from both lineages are superficially indistinguishable (Fig. 2).

With two parallel transects, one on the coast and one in the transitional forest, we explore the transition between mtDNA lineages and relate these to quantitative traits known to be under selection (colour, body dimensions, markings and scale counts, Malhotra & Thorpe 1991; Ogden & Thorpe 2002; Thorpe *et al.* 2005). We also measure climatic and habitat data along the transects in order to give an insight into the nature of selection (convergent or divergent) influencing populations on either side of the
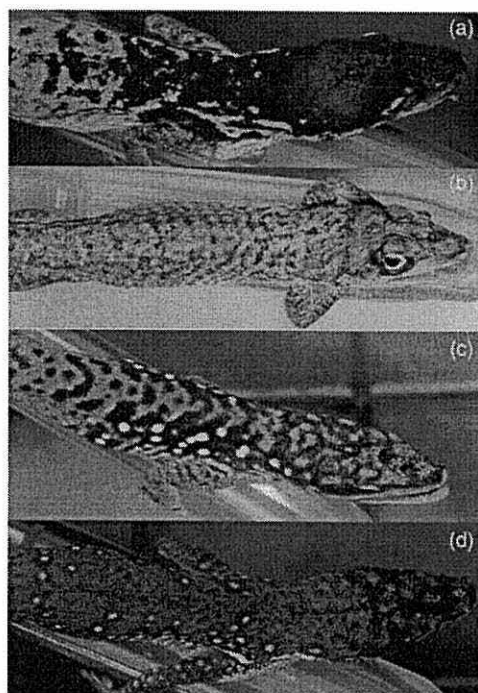
Fig. 2 Marking patterns in *Anolis roquet* from northeastern Martinique. a) Northern coastal form (northwestern lineage) with black head, black cloak and light spots on a green background. b) Southern coastal form (central lineage). Black markings and light spots are entirely absent and lizards are brown. c)–d) Typical lizards from transitional forest habitat (both lineages); black markings and light spots are present to varying degrees, background colour is green.

Fig. 3 Map showing the position of the transects. The townships of Basse-Pointe and Lorrain are included for geographical reference, and are represented by unfilled circles. Inset shows the island of Martinique; study area is boxed.

contact zone. The estimation of the position of mtDNA and quantitative traits cline centres, and their respective widths, allows us to gain insight into the formation and maintenance of the secondary contact zones (Leache & Cole 2007). The influence of allopatry and natural selection on quantitative traits variation also is discussed.

Methods

Sampling

Eight localities along the coast and eight localities in transitional forest habitat were sampled along two linear parallel transects approximately 15 km in length (Fig. 3). The average distance between transect was approximately 6 km. Transects were designed to traverse the boundary between NW and C lineages of *Anolis roquet* as described
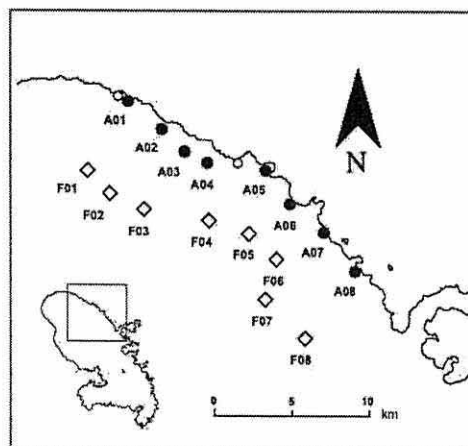
by Thorpe & Stenson (2003). Forty-eight lizards from each locality were hand caught and sampled for DNA (auto-tomised tail tips). Tail tips were stored in tubes containing absolute ethanol for subsequent genetic analysis.

Diagnostic polymerase chain reaction–restriction fragment length polymorphism assignment

We designed a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay to quickly and confidently assign individuals to their mitochondrial clade without the need to sequence them. To do this, we aligned all the cytochrome *b* sequences published by Thorpe & Stenson (2003) and examined them for fixed differences between clades that corresponded to restriction sites. The restriction enzyme *Ssp*I was found to cut the cytochrome *b* fragment at position 160 in the NW clade, but not in the C clade, and was subsequently used to distinguish the two clades. A total of 765 DNA samples were extracted from collected tail tips using QIAGEN DNeasy Blood & Tissue Kit (QIAGEN). A 1063-bp fragment of the cytochrome *b* gene was amplified using the primers MtA-S (5′-ATCTCAGCATGATGAAACTTCG-3′) and MtF-S (5′-TTTGGTTTACAAGACCAATG-3′) in 10-μL reactions using 5 ng of template DNA, 3 mM $MgCl_2$, 0.1 mM of each nucleotide, 0.4 μM of each primer, 0.5 U of *Taq* DNA polymerase (Promega), and 10× buffer (50 mM KCL; 10 mM Tris-HCl, pH 9.0). PCRs were performed using a profile of denaturation of 5 min at 95 °C, followed by 34 cycles (20 s for 95 °C, 30 s at 48 °C, and 45 s at 72 °C) with a

final extension period of 5 min at 72 °C. After amplification, a mixture of 0.2 μL *Ssp*I (New England Biolabs), 2 μL buffer 2 (NEB) and 7.8 μL H$_2$O was added and the samples were digested for 3 h at 37 °C. The reaction was stopped by denaturation of the enzyme at 85 °C for 15 min. Products were visualised on 1.5% agarose gels stained with ethidium bromide under UV illumination, and scored from photographs.

Quantitative trait variation

A subset of 10 adult males from each locality were subject to quantitative trait analysis of the following 20 characters from four character sets: (i) body dimensions: snout to vent length (SVL) jaw length (JL), head length (HL), head depth (HD), head width (HW), upper hindleg length (ULL), lower hindleg length (LLL), dewlap length (DL); (ii) scale counts: postmental (PSC), supra-orbital semicircle (SSC), dorsal (DSC) and ventral (VSC); (iii) markings: number of light patches on head (LPH), number of light patches on anterior body (LPA), number of light patches on posterior body (LPB), percentage of black hood covering head (HEAD), percentage of black cloak covering anterior body (CLOAK). Before recording body measurements and marking patterns, lizards were photographed in standardised light conditions with a Canon EOS 350D fitted with a 100-mm Canon macro lens and a Macro Twin Lite MT-24EX flash. Body dimension measurements were taken in millimetres using electronic digital callipers (Linear Tools), accurate to two decimal points. Photographs were used to confirm scoring of marking patterns, to perform the ventral and dorsal scale counts and were also manipulated in Photoshop (Adobe Systems) to extract the fourth character set; a measure of hue based on the relative proportion of green, red and blue pixels within a standardised area on the dorsal trunk, just behind the front legs. Earlier spectrophotometric analysis of populations in this area (Thorpe & Stenson 2003) indicated that this was an acceptable procedure for dealing with the hue variation of this part of the body when using large samples.

Quantitative traits were analysed independently for each transect. Unless stated, variables were normally distributed. Body dimensions were adjusted against snout-vent length by analysis of covariance (ANCOVA). For each transect, five categorical markings data and two scale counts (SSC and PSC) that had distributions that violate the assumptions of canonical variate analysis (CVA) were entered into a principal components analysis (PCA). The seven components did not violate these assumptions and were inputted into subsequent CVAs as in Dunteman (1989), Zhao *et al.* (1998), Macedonia (2001), Weisrock *et al.* (2005) and Stein & Uy (2007). Hence, we inputted the seven principal components, adjusted body dimensions, two raw scale count variables (DSC and VSC) and two hue variables

(red and green) into a canonical variate analysis using SPSS version 14, to study the variation along the transects.

Climate data

The climate in the northeast Martinique is characterised by moist trade winds that come in from the Atlantic Ocean and hit the slopes of the Pitons du Carbet and Mount Pelée a few kilometres inland. Climate changes rapidly with increasing elevation towards the interior of the island with increased precipitation and lower temperatures in the mountains. Conditions also change with latitude towards the northern and southern extremities of the island. A large body of evidence (see above) indicates that Lesser Antillean anoles adapt by natural selection to this environmental zonation. To elucidate how environmental conditions, and hence selection regimes, varied along the transects, we carried out a PCA (normalised data) on altitude [Institut Géographique National, Carte de Randonée 4502 MT and 4503 MT (French national 1:25 000 resolution maps)], habitat type (Lassere 1979), and three climatic variables, (annual mean temperature, annual precipitation and precipitation seasonality data) from www.worldclim.com. Habitat types were given a nominal code for the analysis. For both transects, the first and second principal components were correlated to the spatial position of the localities along the transect.

Cline fitting

We fitted tanH clines to mtDNA frequency and quantitative trait data using the Fit 1D cline in the program Analyse 1.3 (Barton & Baird 1999: www.biology.ed.ac.uk/research/institutes/evolution/software/Mac/Analyse/Version1.3.html.). The program fits tanH curves to cline data using four variables: cline width, cline centre, and P_{min}–P_{max} (where P_{min} and P_{max} denote minimum and maximum gene frequencies in the tail end of a cline), using a Metropolis algorithm. Normally distributed phenotypic data can be fitted to clines either as single characters or from composite variables (e.g. PCA or CVA scores) where P_{min} and P_{max} are the maximum and minimum character scores in the tails of the cline (Bridle *et al.* 2001; Brumfield *et al.* 2001; Dasmahapatra *et al.* 2002; Takami & Suzuki 2005; Leache & Cole 2007).

Model parameters for mtDNA and quantitative trait clines were estimated independently for both transects, allowing centre and width to vary, fixing P_{min} and P_{max} at 0 and 1, respectively, over 2000 iterations along a best-fit axis. CV 1 locality means from the quantitative trait CVA were transformed to a 0–1 scale before cline fitting, and mtDNA data were represented as 0–1 haplotype frequencies at each locality (Takami & Suzuki 2005). Support values were generated using the support values option in Analyse 1.3,

where parameter values within two likelihood units were generated, equivalent to 95% confidence limits. Significant coincidence and concordance between mtDNA and quantitative traits was attained if centre and width values from one cline could be found within the support limits of the other cline, corresponding to 95% confidence limits (Takami & Suzuki 2005).

Results

Group means and variances for each trait and locality can be found in Tables S1 and S2, Supporting information; correlations between characters for each transect can be found in Tables S3 and S4, Supporting information.

Coastal transect

On the coastal transect, the haplotype frequency change shown by the mtDNA PCR–RFLPs was a sharp step; at each extreme locality there was 100% of NW or C haplotype, a further three localities contained only C haplotypes, and in the remaining four localities more than 90% of individuals were of the common haplotype (Fig. 4a). The changeover in quantitative traits mirrored that of the mtDNA, and the total change between extreme localities amounted to 5.5 pooled within-groups standard deviations (Fig. 4b). There was no overlap of CV scores between the two sets of localities (A01–A04, and A05–A08), and there was a strong and significant correlation between quantitative trait locality means and lineage ($r = 0.99$, $P < 0.01$, $N = 8$). Lizards from either lineage were thereby clearly separated by their appearance: lizards belonging to the northwestern linage were green with black markings and light-patches, this contrasted with lizards belonging to the central lineage which were brown, without black markings or light-patches, and with relatively wider heads (Tables S5 and S6, Supporting information). The bioclimatic PC2 indicated a significant linear change in likely selection regime along transect A ($r = 0.92$, $P < 0.001$, $N = 8$) (Table S7, Supporting information), where the south is less seasonal and habitat is dominated by xerophilous vegetation, although PC1 did not change along the transect ($r = 0.17$, $P > 0.68$, $N = 8$). There was also a strong significant correlation between QT pattern and bioclimatic PC2 variation ($r = -0.84$, $P < 0.01$, $N = 8$). Furthermore, both mtDNA and QT clines were narrow with sharp transitions between locality A04 and A05, coinciding with a geological boundary (Fig. 6a). Width and centres coincided and concurred (Table 1).

Transitional forest transect

In the transitional forest transect, mtDNA haplotype frequency change was gradual, with pure NW and C
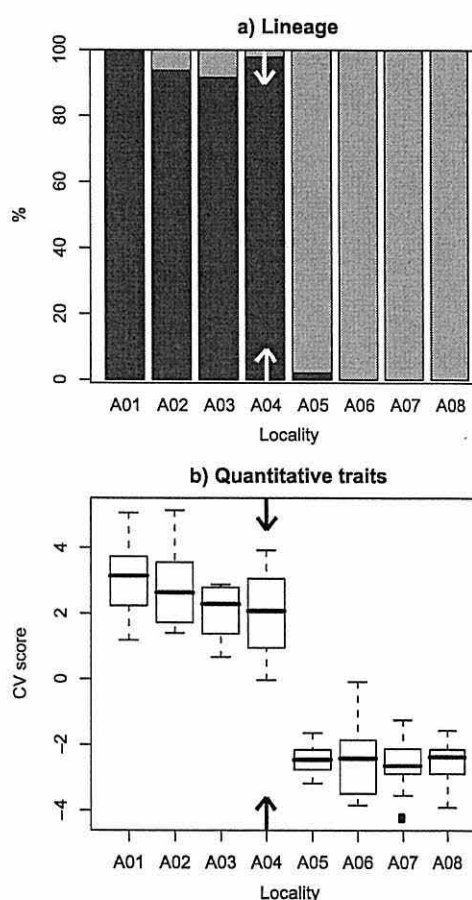
Fig. 4 Lineage distribution and quantitative trait pattern along the coastal transect. (a) Lineage distribution. Dark grey represents northwestern lineage, and pale grey represent central lineage. (b) CV scores for quantitative traits. Haplotype and quantitative trait pattern are similar with sharp, stepped transitions at the geographical boundary (boundary is indicated by arrows).

lineage at the extreme localities (Fig. 5a). The changeover occurred over four localities in the northern part of the transect, and the three southernmost localities were pure C lineage. The quantitative traits showed some variation across the transect; there was a minor gradient between the first and last four localities with 2.2 pooled within-group standard deviations of change between extreme localities (Fig. 5b). In contrast to the coastal transect, QT scores overlapped extensively so no distinct sets could be recognised. However, there was a significant correlation between QT and lineage ($r = 0.79$, $P < 0.02$, $N = 8$). Here,

	Width (m)	Centre position (m)	Log likelihood
Coastal transect			
Mitochondrial DNA	3016 (2549–3587)	6715 (6344–7155)	–16.304
QT	2990 (2057–4044)	6450 (6193–6767)	–0.37
Transitional forest transect			
Mitochondrial DNA	4000 (3171–4893)	5656 (5248–6052)	–2.779
QT	13122 (8868–20518)	9834 (8444–11210)	–12.627

Table 1 Cline widths and centre positions (metres from transect starts) are given for both transects, with best log likelihood for the estimations. Support limits are in parenthesis

extreme northern localities were populated with bright green lizards with black markings, while greenness and markings became less prominent further south and head depths increased (Table S8 and S9, Supporting information). Neither bioclimatic PC1 or PC2 (Table S10, Supporting information) changed significantly along transect F (PC1 $r = -0.43$, $P = 0.29$, $N = 8$: PC2 $r = 0.63$ $P = 0.09$, $N = 8$) and we found no significant correlation between QT pattern and bioclimate (PC1 $r = -0.33$, $P = 0.43$, $N = 8$: PC2 $r = 0.63$, $P = 0.10$, $N = 8$).

Quantitative trait and mtDNA clines had very different centre and widths (Table 1). The QT cline was nearly twice as wide as the mtDNA cline (Fig. 6b). The centre of the mtDNA cline was located between locality F02 and F03, and therefore coincided with the geological boundary, whereas the centre of the QT cline was displaced south.

Discussion

Geological events corresponding with phylogenetic data show evidence of secondary contact of distinct *Anolis roquet* mtDNA lineages on Martinique (Thorpe & Stenson 2003; Thorpe *et al.* 2008). Here we confirmed this relationship on a finer scale and with larger sample numbers; mtDNA transition examined in both transects (identified by the mtDNA cline centre) occurred at the geological boundary where the substrate composition changes from calc-alcali to andesite volcanic rock (Andreieff *et al.* 1976; Maury *et al.* 1990; Sigurdsson & Carey 1991). Broadly speaking, the northwestern precursor island is likely to have joined the central precursor island relatively recently (1–1.5 million years ago), after their respective anole populations had spent an extended period (6–8 million years ago) in allopatry (Thorpe *et al.* 2008). However, more recent volcanic activity of Mount Pelée (500 000–5000 BP) (Traineau *et al.* 1989) may have caused episodes of local extinction and recolonisation. As the transect in the transitional forest was between Mount Pelée and the coastal transect, it is most unlikely that this volcanism could have impacted the coastal region without simultaneously impacting the adjacent transitional forest region, so the timing of secondary contact should be the same in both transects.

Nevertheless, there were notable differences between the two transects despite their proximity and similar history. The wide, flat shape of the QT cline in the transitional forest transect was characteristic of a neutral cline (Barton & Hewitt 1985), suggesting that barriers to gene flow were either absent or very weak in this area. In contrast, the QT cline along the coastal transect showed steepness typical of a cline that was either recently formed, or is maintained by selection (Barton & Hewitt 1985). We can estimate the dispersal rate from the QT clines using the equation $T = 0.35(w/L)^2$ where T is the number of generations necessary to create a cline w metres wide with a rate of L metres of gene flow per generation (Endler 1977). Assuming identical timing of secondary contact in the two transects (see above), the dispersal distance was 4.5 times larger in the transitional forest transect than in the coastal transect. This result strongly suggests restriction of gene flow in the coastal transect.

Endogenous or exogenous selection, or a combination of both, can maintain clines in secondary contact zones long after initial contact (Dasmahapatra *et al.* 2002; Phillips *et al.* 2004). Environmental selection has been shown to be important in anoles and can restrict gene flow (Ogden & Thorpe 2002). Habitat variation (in the shape of a north–south gradient) and QT pattern were correlated on the coastal transect. But compared to the very steep ecotonal transitions in bioclimate and habitat between coast and mountain that drive ecological selection on QTs in *A. roquet* (Ogden & Thorpe 2002; Thorpe *et al.* 2008), the difference in bioclimate and habitat between extreme ends of the coastal transect was relatively small, and the change along the transect was a gentle, linear gradient. Hence, exogenous selection may be a contributing factor, but on its own, it is unlikely to explain the sharp QT transition observed here.

Endogenous selection in secondary contact zones is evidenced by coincidence and concurrence of multiple character clines independent of environmental variation (Brumfield *et al.* 2001; Phillips *et al.* 2004), and occasionally by phenotypic anomalies (Brumfield *et al.* 2001). We found a strong correlation between lineage and QT patterns on the coast, and this finding was also corroborated by the coincidence and concurrence of mtDNA and QT cline
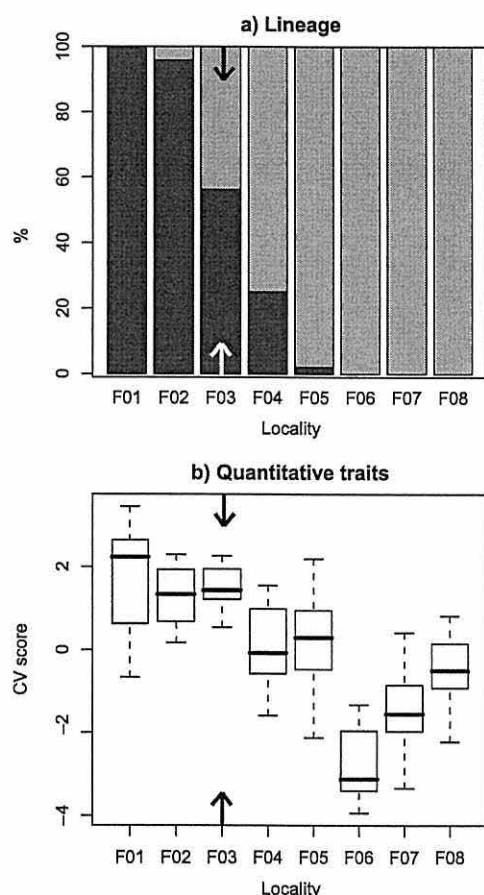
Fig. 5 Lineage distribution and quantitative trait pattern along the transitional forest transect. (a) Lineage distribution. Dark grey represents northwestern lineage, and pale grey represent central lineage. (b) CV scores for quantitative traits. Lineage and quantitative trait variation do not show similar patterns; lineage transition is gradual but coincides with geological boundary (indicated by arrows). Quantitative trait pattern show variation that does not appear to coincide with lineage transition or geological boundary.

Fig. 6 Mitochondrial DNA and quantitative traits clines. Closed squares and thick line represent mtDNA. Open squares and thin line represent quantitative traits. Geological boundary (see discussion) indicated with grey shading. Distance was measured from locality A01 on the coastal transect, and F01 on the transitional forest transect, and projected distance between localities was used. Mitochondrial DNA and quantitative traits clines concur and coincide on the coast (a) but not on the transitional forest transect (b).

centres and widths. Furthermore, at the point where the lineages meet on the coast (locality A05) unusual pigmentation anomalies occur on the head and neck: blotches of white, or skin lacking scales and pigmentation (personal observation). Anomalies of this type were previously described for this region (and this region only) by Lazell (1972); in secondary contact, genetic differences that evolve in allopatry can lead to genic incompatibilities, where

alleles tend to function better in the population they are sourced from, but can nevertheless be functional in a different genetic background (Coyne & Orr 2004). This process can occur with or without natural selection; however, when driven by natural selection, the process is likely to occur more rapidly (Coyne & Orr 2004).

On this relatively fine spatial scale, the QTs on the coast were bimodal which could suggest assortative mating (Jiggins & Mallet 2000). Although it may occur in lizards (Bleay & Sinervo 2007), it is not readily demonstrated and has yet to be unambiguously shown to occur in anoles (Tokarz 1995). Nevertheless, several experimental studies of male dewlap colouration and display behaviour (dewlapping and head bobbing) suggest the possibility of female choice (Crews 1975; Sigmund 1983; Fleishman 1992; Tokarz 1995). Moreover, adaptation to habitat may include changes in dewlap hue to increase visibility/detectability in specific light conditions, which may influence anole interactions (Leal & Fleishman 2002; Leal & Fleishman 2004). Hence, assortative mating as a contributor to the QT pattern merits further investigation.

Results for the coastal transect thus conformed to the expectations of the allopatric speciation model, where differences built up in allopatry are maintained after secondary contact. Both endogenous and exogenous selection may contribute to maintaining the QT cline, and our results are consistent with a strong barrier to gene flow. In contrast, we did not find evidence consistent with barriers to gene flow on the transitional forest transect. Patterns of lineage and QT variation were weakly correlated, but this was not supported by a more detailed analysis of their clines which had different centres and widths. Hence, the transitional forest transect, like the montane rainforest transects (Ogden & Thorpe 2002; Thorpe *et al.* 2008) suggest that the populations do not behave consistently with an allopatric model of speciation. There is overwhelming evidence of the importance of natural selection in shaping population divergence in Lesser Antillean anoles (see references above) and the extent of convergent vs. divergent selection along these two transects may contribute to explain the difference between them. Strong convergent selection in the transitional forest habitat may have eradicated the effects of allopatric divergence, as occurs where these two forms meet in the mountains (Ogden & Thorpe 2002; Thorpe *et al.* 2008). Conversely, the absence of strong convergent selection, and the presence of some divergent selection either side of the secondary contact on the coast, may allow the perpetuation of differences built up in allopatry.

Acknowledgements

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1 Coastal transect locality population means ($\bar{X}$) and variances (σ^2) for all quantitative trait characters. Adjusted values for body dimensions are given. See text for key to abbreviations

Table S2 Transitional transect locality population means ($\bar{X}$) and variances (σ^2) for all quantitative traits characters. Adjusted values for body dimensions are given. See text for key to abbreviations

Table S3 Pearson correlations for each character set of quantitative traits for the coastal transect. Upper triangular half shows scale count and marking pattern correlations, lower triangular half shows raw body dimension correlations. See text for key to abbreviations. *Significant at the 0.05 level. **Significant at the 0.01 level (two-tailed test)

Table S4 Pearson correlations for each character set of quantitative traits for the transitional forest transect. Upper triangular half shows scale count and marking pattern correlations, lower triangular half shows raw body dimension correlations. See text for key to abbreviations. *Significant at the 0.05 level. **Significant at the 0.01 level (two-tailed test)

Table S5 Principal component matrix for markings and scale count data on the coastal transect

Table S6 The first (un-standardised) canonical function coefficient for the coastal transect

Table S7 Principal component matrix for bioclimatic data on the coastal transect

Table S8 Principal component matrix for markings and scale count data on the transitional forest transect

Table S9 The first (un-standardised) canonical function coefficient for the transitional forest transect

Table S10 Principal component matrix for bioclimatic data on the transitional forest transect

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