DOCTOR OF PHILOSOPHY

Microsatellites of Mites in Forensics

Al-Khalify, Amal Mohsan H

Award date:
2019

Awarding institution:
Bangor University

Microsatellites of Mites in Forensics

A thesis submitted for the degree of
Doctor of Philosophy at Bangor University

by

Amal Mohsan H Al-Khalify

School of Natural Sciences
Bangor University, Wales, UK

2018

Dedication

My mother was very ill for a considerable period of time while I was researching and working on my PhD.

She was so proud and pleased that her daughter went to university to study for her PhD. Unfortunately, she never got to see me finish this research as she and my brother passed away in 2017. To honour their memory, I forced myself to come back to complete my PhD.

Therefore, I honour the memory of my mother, Ruqayyah, and my brother, Waleed, in all my life's successes and achievements. I dedicate this work to the memory of them and their love, which will always live in my heart.

Acknowledgment

List of contents

Microsatellites of Mites in Forensics

1 Introduction, aim and objectives

1.1 Forensics and the characterisation of biological traces

At the heart of most forensic work lies the establishment of identity through linking characteristics or characters of any sort with a person, organism or object. This can take the form of linking fingerprints to a person, linking facial characteristics of a picture to a person, linking blood spatter or other bodily fluids to a person, linking hair or bone fragments to a person or linking a probable descendant to its potential parents in paternity analysis. Similarly, the identity of inanimate objects such as fragments of glass might be linked to a sheet of glass, fragments of paint might be linked to an object, or fibres to a piece of clothing or another objects; even the identity of electronic data might be linked to a person. The identity of saliva might be linked to a person or an animal, components of animals might be linked to endangered animals, fragments of wood might be linked to a ladder used in an abduction, or soil mites on discarded clothing or on previously buried paper money might be linked to specific places, just to give a few examples. Wherever biological samples are involved in these examples, the linking of the trace to its origin has most likely be achieved with the use of microsatellites. The identity of traces of human blood, hairs, or sperm and paternity determinations are now almost exclusively obtained with the help of microsatellites.

The application of microsatellites to the analysis and characterisation of the ever-increasing diversity of non-human biological trace samples is still in its infancy. Forensic acarology is currently seeing an expantion as a resource in the forensic arsenal. However, microsatellites have not so far been exploited in forensic biology.

With few exceptions, mites (Acari) are ubiquitous in the human environment and therefore mites should be ubiquitous at many crime scenes and in many trace samples. The very small size of mite species – most mites are not recognized with the naked eye –, and the difficulties encountered in identifying mite species by non-acarologists, might have made mites one of the most overlooked or actively ignored traces in forensics.

1.2 Aim and objectives of the thesis

The aim of the thesis is to contribute to the development of mites as forensic trace evidence. This aim will be approached from several angles. The research in this thesis is at the very beginning of the development of the subject area. As such, it is for the major part descriptive research meant to enable hypothesis-driven research later on. The interrogation of the first genome of humans like the first genome of any species is inherently descriptive. Here, for the first time, the whole genome of a mite species is analysed for microsatellites.

Mites like most organisms are associated with specific environments and habitats of given localities. Forensic biology is put in practice in specific biogeographical regions. The Arabian Peninsula and its adjacent countries have been chosen as the specific biogeographical region. Chapter 2 examines the state of the art of non-human forensic biology in the Arabian Peninsula and its adjacent countries. Importantly, it investigates a critical resource currently available for non-human forensic biology. This resource is access to region specific taxonomic keys for the identification of biological trace organisms. While the thesis sees the future of non-human forensic biology in the molecular realm, any molecular approach will be governed by the primary taxonomic identification of the species diversity. Taxonomic identification depends on the availability of taxonomic keys. One purpose of this chapter is to identify knowledge gaps.

As stated above, mites are ubiquitous, which means forensically important mites can be found in many different habitats, such as a decomposing human corpse, in soil, in plant material, or in dust or house dust. Dust has been chosen as a focus in this thesis. The potential of dust in forensic trace analyses is investigated in Chapter 3.

Microsatellites have been the most successful tool so far in the molecular analysis of human trace evidence. In Chapter 4, the analysis of microsatellites in mites is reviewed.

The first critical step in any molecular analysis of trace evidence is the optimal extraction of DNA. While the extraction of DNA from human blood samples did not encounter any physical barriers because white blood cells are easily lysed for DNA

extraction, PCR inhibitors in red blood cells proved to be challenging in the beginning. Mites on the other end possess very tough exoskeletons made of cuticular material that can also include PCR inhibitors. The exoskeleton of most mite species is so tough that it is normally found in archaeological samples alongside bone fragments. An extraction method employed for forensic purposes also must accommodate very small samples sizes and limit the risk of cross-contamination as much as possible, meaning it should be using single-use, disposable components. The extraction of DNA through bead beating is investigated in Chapter 5.

When dust mites are found in a forensic trace sample, the question arises where did these mites come from; from which specific niche, which house, which apartment or biologically formulated, from which population did these mites originate. The easiest way to answer these questions is to make use of microsatellites. The genome of one of the most common and best-known house dust mite species, *Dermatophagoides farinae*, is investigated for repetitive sequences in Chapter 6. Based on this analysis, over one hundred microsatellites are developed for forensic use.

The development of microsatellites is quite labour intensive. The potential use of microsatellites developed for one species in another species is investigated in Chapter 7. A house dust species related to *D. farinae* is checked for cross-species conservation. While the formulation of this objective suggests a positive situation, the reuse of microsatellites, which is common in an ecological context for the study of identified individuals, it presents itself as pitfall in a forensic context. If a microsatellite is similar or identical in more that one species, it becomes forensically useless. To estimate this risk, the phylogenetically closest sister species to *D. farinae*, *D. pteronyssinus* is scrutinised.

The final step in forensic research is the reporting of the findings and especially of forensically important data in the forensic literature. For example, microsatellites that have not been checked for cross-species conservation might easily end up in a predatory forensic journal without functional peer review. This could likely lead to a miscarriage of justice. To prevent this, an in-depth analysis of research journal covering aspects of forensic sciences and legal medicine has been

undertaken. Chapter 8 culminates with a proposal for a comprehensive white list of journals for forensic biology.

Conclusions of the thesis are summarized in Chapter 9 and used for an outlook into the future of molecular analysis of non-human biological traces.

2 Forensic biology and trace analyses in the Arabian Peninsula and adjacent countries

2.1 History

Forensic expertise originally rested with physicians. One of the first physicians to which a forensic role was ascribed to is Imhotep, 2,667-2,648 BC, who was the physician to Egyptian pharaoh Djoser (Zozer, also known as Tosorthros or Sesorthos) of the 3rd dynasty during the Old Kingdom (Smith, 1951). Imhotep, Figure 2.1, was also the chancellor to the pharaoh, high priest of the sun god Ra, and probably the architect of the step pyramid.

Figure 2.1 – Imhotep in Egypt, more than 4,500 years ago, was likely one of the first forensic practitioners and medical examiners, enquiring about biological processes in a forensic context, suggesting that forensic biology in the widest sense had its cradle in the Arabian world. Picture egyptprivatetourguide.com

Over time, physicians remained acting as expert witnesses in cases where medical expertise was helpful in legal procedures but did gain an official forensic title. In Roman Egypt of 100-400 CE, physicians seem to have taken over more and more specific forensic functions in cases involving major injuries and violent death; and prosphoneseis, a medical report, is set up for the first time and used as evidence in

trails (Amundsen and Ferngren, 1978). Forensic medicine or legal medicine evolved as a separate medical sub-discipline in its own right.

The Egyptian forensic tradition was revived when in 1862 Ibrahim Pasha Hassan went to France and Austria for training in forensic medicine. Later, Hasan started teaching forensic medicine as an independent subject at the School of Medicine of Eini Palace. In 1917, a special chair in forensic medicine was erected at the Faculty of Medicine at the University in Cairo. The chair was held by Sydney Smith for 11 years, followed by John Glaister until 1932, when Mahmoud Beck Maher became chief senior doctor of the newly established Forensic Medicine Authority in Egypt (Kharoshah et al., 2011).

In Iraq, a medicolegal department was added to the Central Medical Laboratory. Forensic medicine became part of the curriculum of the College of Medicine in Baghdad in 1972 and is now part of all six medical schools in the country (Ali, 1980).

Forensic medicine started in the Arabian Peninsula in the nineteen sixties. A Royal Degree in 1962 established forensic medicine centres by the Ministry of Health of Saudi Arabia (Al Madani et al., 2012). At the time, forensic medicine started in other Arab countries like Tunisia as well (Mghirbi et al., 2004). The development in Lebanon and in Palestine are described by Oueidat et al. (2012) and Al Madani et al. (2012); (Alkahtani et al., 2015; Akcan and Yildirim, 2016); Daher-Nashif (2017).

The medicolegal system in the Arabian Gulf states is different from the majority of Muslim countries. Particularly in Saudi Arabia, criminal and civil judiciary is solely based on Shari'ah law, whereas most Muslim countries have a combination of Shari'ah and statute law (Al Madani et al., 2012; Alkahtani et al., 2015; Akcan and Yildirim, 2016; Meilia et al., 2018). For example, autopsies are much less common in Saudi Arabia than elsewhere (Al Madani et al., 2012; Mohammed and Kharoshah, 2014; Al-Waheeb et al., 2015; Madadin, 2015; Bamousa et al., 2016; Sajid, 2016; Meilia et al., 2018). Yet, Egyptians were one of the first civilisations that practiced autopsy and introduced the forensic examination of internal organs of humans (Kharoshah et al., 2011).

Specialisation of forensic medicine in the Arab states focussed initially on forensic psychiatry on one side and forensic genetics on the other side (Touari et al., 1993; Chaleby, 1996), establishing allele frequencies in local populations (Ota et al., 1997; Tahir et al., 1997; Klintschar et al., 1998). Other disciplines like forensic nursing (Alsaif et al., 2014) or forensic dentistry (Al Sheddi and Al Asiri, 2015) followed.

To serve Arab law enforcement agencies, Naïf Arab University for Security Sciences (NAUSS) was established in 1978 in Riyadh, Saudi Arabia, as an intergovernmental institution lead under the aegis of the Council of Arab Ministers of Interior. The University's research does currently not involve non-human forensic biology.

2.2 Forensic biology

The term forensic biology is currently undergoing a change in meaning. Originally, forensic biology denoted only what is now better known as human forensic genetics, the analysis of human DNA, sometimes referred to as forensic DNA typing. Now, forensic biology encompasses more and more the non-human biology in forensics. Two current text books at opposite ends of the spectrum illustrate this transition; the book *Forensic Biology* by Richard Li originally was exclusively human based, but has now a 4-page subsection on Forensic Entomology among 567 pages in its second edition (Li, 2015); whereas more than half of the book *Essential Forensic Biology* by Alan Gunn in its third edition is covering everything from Invertebrates, via Wildlife Forensics, to Plants, Protists, Fungi and Microbes (Gunn, 2019).

Forensic evaluation of homicidal snake bites, acarology in crimino-legal investigations, or soil bulb mites as trace evidence for the location of buried money are just three examples, showing the addition of non-human biological traces to current forensic biology (Paulis and Faheem, 2016; Hani et al., 2018; Perotti and Braig, 2019).

2.3 Forensic entomology

The first succession studies of arthropods on exposed rabbit carcasses were conducted in Alexandria, Egypt, in 1988 and 1989, comparing decomposition during

all four seasons (Tantawi et al., 1996). The study of Tantawi et al. (1996) on rabbit carcasses not only analysed insects but also included mites (Acari). The entomofauna of decomposing tilapia fish and desert snails in both, pitfall and hanging traps was studied in in the western desert of Egypt (Hegazi et al., 1991). Rabbit decomposition studies were continued for Nasr City, Egypt, including dogs (Zeariya et al., 2015), Upper Egypt including indoors (Aly et al., 2017), north-eastern Egypt including rats (Aly et al., 2013), Kuwait (Al-Mesbah et al., 2012), An-Najaf province and Al Kufa City of Iraq (Albushabaa, 2016; Albushabaa and Almousawy, 2016), and for the mountains of Al-Baha Province of southwestern part of Saudi Arabia (AbouZied, 2014), Al-Ahsaa Oasis (Shaalan et al., 2017), urban, desert, and agricultural sites in Riyadh (Mashaly and Al-Mekhlafi, 2016) and including ants (Mashaly et al., 2018a), the west of Iran (Salimi et al., 2018a), indoor rat carcases (Keshavarzi et al., 2015a), and with burnt rabbit carcasses for Riyadh (Mashaly, 2016). Studies with guinea pig carcasses have been performed in Benha City, Egypt (Ibrahim et al., 2013) and pig carcasses in Badghan-Mount, Lebanon (Shayya et al., 2018c). The impact on insect succession on rabbits partially or fully submerged in tap water positioned roughly one meter above the ground was studied in Riyadh (Haddadi et al., 2019). The effect of clothing on rabbit on insect succession on rabbit carcasses was determined (Mashaly et al., 2019). Additional rabbit decomposition studies have been performed for the oasis of Al-Ahsaa in eastern Saudi Arabia (Shaalan et al., 2017) and for the succession of carrion beetles in different habitats in Riyadh (Mashaly, 2017). Prevalence studies have been conducted for Iran (Haghi et al., 2017). First reports of the blow fly *Chrysomya megacephala* come from Algeria (Taleb et al., 2018). Ten species of blow flies new to science have been reported from Tunisia, the Sinai Peninsula and adjacent areas (Rognes, 2002).

Life cycle parameters of local blow and flesh flies are important for estimations of time of death. Development rates for the flesh fly *Parasarcophaga (Liopygia) ruficornis* (Diptera, Sarcophagidae) have been determined in Riyadh (Amoudi et al., 1994), for the blow fly *Chrysomya albiceps* in Baghdad (Augul and Jassim, 2009), Jeddah (Al-Shareef and Al-Qurashi, 2016; Al-Shareef et al., 2016), for C. *albiceps* and *Calliphora vicina* (Salimi et al., 2018d), and including flesh flies (Shiravi

et al., 2011). The biological effect or entomotoxicology of cadmium on larvae of the blow fly *C. albiceps* (Diptera: Calliphoridae) has been investigated using ground beef (Al-Misned, 2001, 2003); morphine could be recovered from larvae of *C. albiceps* feeding on rabbit carcases (Salimi et al., 2018b; Salimi et al., 2018c). The influence of organophosphate insecticide pirimiphos-methyl on insect colonisation succession was studied with rabbit carcasses (Abd El-Bar and Sawaby, 2011). The effect of the opioid tramadol on the developmental rate of necrophagous flies was studied with the help of rat carcasses (AbouZied, 2016). Human wound myiasis caused by *Phormia regina* (Diptera: Calliphoridae) and *Sarcophaga haemorrhoidalis* (Diptera: Sarcophagidae) has been reported from Egypt (Abdel-Hafeez et al., 2015).

Life cycle information for *Creophilus maxillosus* (Coleoptera: Staphylinidae) (Keshavarzi et al., 2015b) and *Dermestes frischii* (Coleoptera: Dermestidae) (Keshavarzi et al., 2015c) on a human corpse is available from Iran, and on forensically important Dermestidae beetles from Egypt (Azab et al., 1973).

Possibly the first application of forensic entomology in Arab countries comes from Kuwait. Third instar larvae of *Parasarcophaga ruficornis* were used to estimate the minimum post-mortem interval for a corpse in an air-conditioned room. Secondary colonizer was *C. albiceps* (Al-Mesbah et al., 2011). Reports of necrophagus insects collected from human corpses are now available from Assuit, Egypt using left-over parts after surgery (Galal et al., 2009); from whole bodies from Riyadh (Alajmi et al., 2016), Jeddah (Al-Shareef and Zaki, 2017), Saudi Arabia; from Shiraz, Iran (Moemenbellah-Fard et al., 2015; Keshavarz et al., 2016; Moemenbellah-Fard et al., 2018). Alajmi et al. (2016) mention the implementation of a Saudi database of forensically relevant insects.

Increasingly, forensically important insects are identified by sequencing gene fragments (cytochrome oxidase I and II) of the insect's mitochondria, for flies (Aly and Wen, 2013a, b; Aly, 2014; Salem et al., 2015; Alajmi et al., 2016; Mgashaly et al., 2017; Shayya et al., 2018a) and for beetles (Alajmi et al., 2016; Mashaly et al., 2018b). Mashaly et al. point out that the standard Folmer region of cytochrome oxidase I used for barcoding is not sufficient to differentiate between *Musca* species (Mashaly et al., 2017). The Cyt b-tRNAser-ND1 region of the mitochondrion did in a Lebanese

study of *Calliphora* and *Lucilia* species by Shayya et al. (2018a) not show the resolution expected from the European study by GilArriortua et al. (2013), Table 2.1.

Molecular identification of forensically important fly species

Species	COI 658 bp	Cyt b-tRNAser-ND1 ≥ 495 bp	ITS2 ≥ 310 bp
C. vicina	√ 0.2	0.1	√ 0.9
C. vomitoria	√ 0	0	√
Ch. albiceps	√ 0.2	0.1	√ 0
L. caesar	√ 0.4	0.3	– 0
L. cuprina	–		√
L. illustris			–
L. sericata	– 0	0.1	√ 0

Table 2.1 – Ability of particular gene fragments to identify forensically important fly species molecularly (Shayya et al., 2018a). A marker that is able to identify a species unambiguously is denoted with a check mark, the failure with a dash. Values in table, intraspecific divergences in %, where known locally.

2.4 Wildlife forensics

Microsatellites were developed for the Arabian oryx that could be applicable in a forensic context (Marshall et al., 1999).

Instead of using microsatellites for the animals themselves that are involved in illegal trade, researchers have developed microsatellites for mite species parasitic on the animals being traded to determine the origin of the wildlife. Applied to wildebeest or genus (*Connochaetes* spp) imported by the United Arab Emirate from Tanzania, the origin of the infestation could be determined (Alasaad et al., 2009; Alasaad et al., 2012).

2.5 Keys

One of the important resources in forensic entomology are local keys to the species of forensic importance, Table 2.2. Many of these publications are not easily retrievable with scientific search engines.

Keys and check lists to forensically important species

Species	Country	Reference
1. Diptera		
Blow flies, Calliphoridae	Middle East	Akbarzadeh et al., 2015; El-Hawagry and El-Azab, 2019
	Egypt	Shaumar et al., 1989
	Oman	Deeming 1996
	Saudi Arabia	Büttiker et al., 1980; Dawah and Abdullah, 2009b; Setyaningrum and Al Dhafer, 2014
Flesh flies, Sacrophagidae	Egypt	Shaumar and Mohammed, 1983; El-Hawagry and El-Azab, 2019
House and stable flies, Muscidae, Fannidae	Saudi Arabia	Dawah and Abdullah, 2009a
Hump-backed or Scuttle flies, Phoridae	Saudi Arabia	Disney 2006, 2009
Synanthropic flies	Saudi Arabia	Büttiiker et al. 1979
2. Coleoptera		
	Palaearctic	Diaz-Aranda et al., 2018
	Egypt	Alfieri, 1976; Sawaby et al., 2016
Woodworm beetles, Anobiidae	Saudi Arabia	Español 1979, 1981
Ground beetles, Carabidae	Saudi Arabia	Basilewsky, 1979; Heinertz, 1979; Mateu, 1979; Mandl, 1980; Nagel, 1982; Mandl, 1986; Mateu, 1986
Bone beetles, Cleridae	Saudi Arabia	Winkler 1981; Menier 1986
Skin beetles, Dermestidae	Arabian Peninsula	Háva, 2011, 2015
	Saudi Arabia	Mroczkowski, 1979, 1980
Clown beetles, Histeridae	Lebanon	Shayya et al., 2018b
	Saudi Arabia	Kryzhanovskij 1979
Sap beetles, Nitidulidae	Saudi Arabia	Mroczkowski 1979; Jelínek 1988
Carrion beetles, Silphidae	Saudi Arabia	Schawaller 1981
Rove beetles, Staphylinidae	Saudi Arabia	Coiffait 1979; Puthz 1980; Coiffait 1981
Darkling betles, Tenebrionidae	Saudi Arabia	Kaszab 1979; Kaszab 1981; Kwieton 1981; Kaszab 1982
Hide beetles, Trogidae	Saudi Arabia	Scholtz 1980
3. Insects		
	Egypt (in tombs)	(Huchet, 1996)
	Iraq	Derwesh, 1965
	Saudi Arabia	(Al-Ahmadi and Salem, 1999)

4. Acari		
Mites, Acariformes	Saudi Arabia	Samšiňák 1979; Bader 1980; Bayoumi and Al-Khalifa 1986
Ticks, Parasitiformes	Saudi Arabia	Hoogstraal et al., 1981; Hoogstraal, 1982; Hoogstraal and Bafort, 1982; Hoogstraal et al., 1983b, a; Bafort and Fain, 1985; Hoogstraal, 1985; Hoogstraal et al., 1985; Al-Khalifa et al., 2006; Diab et al., 2006
	Yemen	Ueckermann et al., 2006

Table 2.2 – References to the keys to taxa of forensic importance.

2.6 Conclusions

The Arabian countries show an impressive body of research and resources in forensic entomology. Most of the entomological work is descriptive in terms of biodiversity, morphologically as well as molecularly, and of factors that might interfere with the entomofauna. Increasingly, insects are used for estimating post mortem intervals. Investigating and interrogation organisms as traces has yet to come. Many of the smaller subdisciplines of forensic biology like forensic acarology, botany, microbiology, palynology are still awaiting their place in the national forensic systems.

Big challenges lie ahead. For forensic laboratories and agencies, this might be in the form of international certification, for example ISO/IEC 17020 concerning impartiality, independence, and confidentiality or ISO/IEC 17025 concerning measurement uncertainty, traceability, and analytical validation.

For some countries, the biggest challenge of integrating forensic sciences into their legal system might be Shari'ah law (Alkahtani et al., 2015). Forensic biology in the form of forensic entomology might provide solutions for estimating the time of death where interventions on the body itself are prohibited by religious views (Gatrad, 1994; Bhootra, 2005; Mohammed and Kharoshah, 2014; Aljerian et al., 2015; Madadin, 2015; Akcan and Yildirim, 2016; Al-Saif et al., 2016; Bamousa et al., 2016; Madadin et al., 2017).

The danger of predatory publishing has not yet been realized.

2.7 References

Threat from predatory publishing

As far as the references in this review are concerned, the integrity of the peer review process is in doubt for the following journals

- *Advances in Environmental Biology*

  Al-Shareef et al. (2016)

- *Life Science Journal*

  Sawaby et al. (2016)

- *IOSR Journal of Pharmacy and Biological Sciences*

  Aly et al. (2017)

- *International Journal of Forensic Science and Pathology*

  Keshavarzi et al. (2015b); Keshavarzi et al. (2015c); Keshavarz et al. (2016)

- *Journal of American Science*

  Al-Shareef and Zaki (2017)

- *Journal of Entomology and Zoology Studies*

  Keshavarzi et al. (2015a); Moemenbellah-Fard et al. (2015); Zeariya et al. (2015); Haghi et al. (2017)

- *Research Journal of Pharmaceutical Biological and Chemical Sciences*

  Albushabaa (2016); Albushabaa and Almousawy (2016)

Abd El-Bar MM and Sawaby RF (2011) A preliminary investigation of insect colonization and succession on remains of rabbits treated with an organophosphate insecticide in El-Qalyubiya Governorate of Egypt. *Forensic Science International* 208: E26-E30.

Abdel-Hafeez EH, Mohamed RM, Belal US, Atiya AM, Takamoto M and Aosai F (2015) Human wound myiasis caused by *Phormia regina* and *Sarcophaga haemorrhoidalis* in Minia Governorate, Egypt. *Parasitology Research* 114: 3703-3709.

AbouZied EM (2014) Insect colonization and succession on rabbit carcasses in southwestern mountains of the Kingdom of Saudi Arabia. *Journal of Medical Entomology* 51: 1168-1174.

AbouZied EM (2016) Postmortem attraction of sarcosaprophagous Diptera to tramadol-treated rats and morphometric aspects of the developed larvae. *Neotropical Entomology* 45: 326-332.

Akbarzadeh K, Wallman JF, Sulakova H and Szpila K (2015) Species identification of Middle Eastern blow flies (Diptera: Calliphoridae) of forensic importance. *Parasitology Research* 114: 1463–1472.

Akcan R and Yildirim MS (2016) Forensic medicine and science applications in Islamic law. *Journal of Forensic and Legal Medicine* 42: 11-12.

Al Madani OM, Kharoshah MAA, Zaki MK, Galeb SS, Al Moghannam SA and Moulana AAR (2012) Origin and development of forensic medicine in the Kingdom of Saudi Arabia. *American Journal of Forensic Medicine and Pathology* 33: 147-151.

Al Sheddi M and Al Asiri A (2015) Awareness of the scope and practice of forensic dentistry among dental practitioners. *Australian Journal of Forensic Sciences* 47: 194-199.

Al-Ahmadi AZ and Salem MM (1999) *Entomofauna of Saudi Arabia, General Survey of Insects Reported in the Kingdom of Saudi Arabia. Part I: Checklist of Insects*. King Saud University Press, Riyadh.

Al-Khalifa MS, Diab FM, Al-Asgah NA, Hussein HS and Khalil GA (2006) Ticks (Acari: Argasidae, Ixodidae) recorded on wild animals in Saudi Arabia. In: Krupp F (ed) *Fauna of Arabia*, vol 22. Karger, Basel; pp 225-232.

Al-Mesbah H, Al-Osaimi Z and El-Azazy OME (2011) Forensic entomology in Kuwait: The first case report. *Forensic Science International* 206: E25-E26.

Al-Mesbah H, Moffatt C, El-Azazy OME and Majeed QAH (2012) The decomposition of rabbit carcasses and associated necrophagous Diptera in Kuwait. *Forensic Science International* 217: 27-31.

Al-Misned FAM (2001) Biological effects of cadmium on life cycle parameters of *Chrysomya albiceps* (Wiedemann) (Diptera : Calliphoridae). *Kuwait Journal of Science & Engineering* 28: 179-188.

Al-Misned FAM (2003) Effect of cadmium on the longevity and fecundity of the blowfly *Chrysomya albiceps* (Wiedemann) (Diptera : Calliphoridae). *Kuwait Journal of Science & Engineering* 30: 81-94.

Al-Saif DM, Al-Faraidy MA, Madadin MS, Al-Bayat MI, Al-Sowayigh KS, Al-Shamsi GA, Aldossary MR, Al-Madani OM and Kharoshah MA (2016) The attitude of people with an Arabic Islamic cultural background toward medico-legal autopsy. *Australian Journal of Forensic Sciences* 48: 557-563.

Al-Shareef LAH and Al-Qurashi SID (2016) Study of some biological aspects of the blowfly *Chrysomya albiceps* (Wiedemann 1819) (Diptera: Calliphoridae) in Jeddah, Saudi Arabia. *Egyptian Journal of Forensic Sciences* 6: 11-16.

Al-Shareef LAH, Zaki MK, Ghannam AA, Alweail ZO and Al Garni AB (2016) Estimation of postmortem interval for human corpse using the blowfly *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae) in Jeddah, Kingdom of Saudi Arabia. *Advances in Environmental Biology* 10: 245-249.

Al-Shareef LAH and Zaki AM (2017) Arthropods associated with human remains and determination of postmortem interval in Jeddah, Kingdom of Saudi Arabia. *Journal of American Science* 13: 106-114.

Al-Waheeb S, Al-Kandary N and Aljerian K (2015) Forensic autopsy practice in the Middle East: Comparisons with the west. *Journal of Forensic and Legal Medicine* 32: 4-9.

Alajmi RA, Farrukh A, Aljohani H and Mashaly AMA (2016) First report of necrophagous insects on human corpses in Riyadh, Saudi Arabia. *Journal of Medical Entomology* 53: 1276-1282.

Alasaad S, Soglia D, Spalenza V, Maione S, Soriguer RC, Perez JM, Rasero R, Degiorgis MPR, Nimmervoll H, Zhu XQ and Rossi L (2009) Is ITS-2 rDNA suitable marker for genetic characterization of *Sarcoptes* mites from different wild animals in different geographic areas? *Veterinary Parasitology* 159: 181-185.

Alasaad S, Schuster RK, Gakuya F, Theneyan M, Jowers MJ, Maione S, Min AM, Soriguer RC and Rossi L (2012) Applicability of molecular markers to determine parasitic infection origins in the animal trade: a case study from *Sarcoptes* mites in wildebeest. *Forensic Science, Medicine, and Pathology* 8: 280-284.

Albushabaa SHH (2016) Insect succession and decomposition of buried rabbits during two seasons in Al Kufa City, Iraq. *Research Journal of Pharmaceutical Biological and Chemical Sciences* 7: 2976-2985.

Albushabaa SHH and Almousawy HR (2016) Insect succession and carcass decomposition during spring and summer in An-Najaf province-Iraq. *Research Journal of Pharmaceutical Biological and Chemical Sciences* 7: 2455-2464.

Alfieri A (1976) The Coleoptera of Egypt. *Mémoires de la Société Entomologique d'Égypte.* 5: 1- 361.

Ali WW (1980) The establishment and practice of forensic medicine in Iraq. *American Journal of Forensic Medicine and Pathology* 1: 81-84.

Aljerian K, Alhawas A, Alqahtani S, Golding B and Alkahtani T (2015) First virtual autopsy in Saudi Arabia: A case report with literature review. *Journal of Forensic Radiology and Imaging* 3: 76-79.

Alkahtani T, Aljerian K, Golding B and Alqahtani S (2015) Forensic science in the context of Islamic law: A review. *Journal of Forensic and Legal Medicine* 34: 179-181.

Alsaif DM, Alfaraidy M, Alsowayigh K, Alhusain A and Almadani OM (2014) Forensic experience of Saudi nurses; an emerging need for forensic qualifications. *Journal of Forensic and Legal Medicine* 27: 13-16.

Aly MZY, Osman KSM, Galal FH and Ali GHM (2017) Comparative study on outdoor and indoor forensic insects encountered on rabbit corpses in Upper Egypt. *IOSR Journal of Pharmacy and Biological Sciences* 12: 41-54.

Aly SM and Wen J (2013a) Molecular identification of forensically relevant Diptera inferred from short mitochondrial genetic marker. *Libyan Journal of Medicine* 8: 20954.

Aly SM and Wen J (2013b) Applicability of partial characterization of cytochrome oxidase I in identification of forensically important flies (Diptera) from China and Egypt. *Parasitology Research* 112: 2667-2674.

Aly SM, Wen J, Wang X, Cai J, Liu Q and Zhong M (2013) Identification of forensically important arthropods on exposed remains during summer season in northeastern Egypt. *Journal of Central South University (Medical Sciences)* 38: 1-6.

Aly SM (2014) Reliability of long vs short COI markers in identification of forensically important flies. *Croatian Medical Journal* 55: 19-26.

Amoudi MA, Diab FM and Aboufannah SSM (1994) Development rate and mortality of immature *Parasarcophaga (Liopygia) ruficornis* (Diptera, Sarcophagidae) at constant laboratory temperatures. *Journal of Medical Entomology* 31: 168-170.

Amundsen DW and Ferngren GB (1978) Forensic role of physicians in Ptolemaic and Roman Egypt. *Bulletin of the History of Medicine* 52: 336-353.

Augul RS and Jassim SY (2009) Study of some biological and ecological aspects of the fly *Chrysomya albiceps* (Wiedemann) (Diptera; Calliphoridae). *Journal of Al-Anbar University for Pure Science* 3: 1-4.

Azab AK, Tawfik MFS and Abouzeid NA (1973) Factors affecting the rate of oviposition in *Dermestes maculatus* De Geer (Coleoptera: Dermestidae). *Bulletin de la Société Entomologique d'Égypte* 56: 49-59.

Bader C (1980) Hydrachnellae: Fam. Nilotoniidae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 2. Karger, Basel; pp 49-56.

Bafort JM and Fain A (1985) Hypopi of Hypoderidae (Acarina: Sarcoptiformes) parasites of the white pelican in the Arabian Gulf, Saudi Arabia. In: Büttiker W and Krupp F (eds) *Fauna of Saudi Arabia*, vol 6. Karger, Basel; pp 162-164.

Bamousa MS, Al-Fehaid S, Al-Madani O, Al Moghannam S, Galeb S, Youssef M and Kharoshah MAA (2016) The Islamic approach to modern forensic and legal medicine issues. *American Journal of Forensic Medicine and Pathology* 37: 127-131.

Basilewsky P (1979) Coleoptera: Fam. Carabidae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 1. Karger, Basel; pp 141-146.

Bayoumi BM and Al-Khalifa MS (1986) Oribatid Mites (Acari) of Saudi Arabia. In: Büttiker W and Krupp F (eds) *Fauna of Saudi Arabia*, vol 7. Karger, Basel; pp 66-92.

Bhootra B (2005) Forensic pathology services and autopsy law of Qatar. *Journal of Clinical Forensic Medicine* 13.

Büttiker W, Attiah MD and Pont AC (1979) Diptera: Synanthropic flies. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 1. Karger, Basel; pp 352-367.

Büttiker W, Habayeb S and Zumpt F (1980) First records of the tumbu fly (*Cordylobia anthropophaga* [Blanchard]), (Diptera: Fam. Calliphoridae). In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 2. Karger, Basel; pp 440-443.

Chaleby KS (1996) Issues in forensic psychiatry in Islamic jurisprudence. *Bulletin of the American Academy of Psychiatry and the Law* 24: 117-124.

Coiffait H (1979) Coleoptera: Fam. Staphylinidae, Subfam. Xantholinae, Staphylininae, Paederinae, Oxytelinae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 1. Karger, Basel; pp 162-180.

Coiffait H (1981) Coleoptera: Fam. Staphylinidae, Subfam. Xantholininae, Staphylininae, Paederinae, Oxytelinae, Aleocharinae (Part 2). In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 3. Karger, Basel; pp 236-242.

Daher-Nashif S (2017) Historical and present-day practices of forensic medicine in Palestine: Body, society, and science. *Jerusalem Quarterly* 70: 75-96.

Dawah HA and Abdullah MA (2009a) The Muscidae (Diptera: Brachycera: Muscomorpha) of south-western Saudi Arabia. In: Krupp F (ed) *Fauna of Arabia*, vol 24. Karger, Basel; pp 373-396.

Dawah HA and Abdullah MA (2009b) The Calliphoridae (Diptera: Cyclorrhapha) of south-western Saudi Arabia. In: Krupp F (ed) *Fauna of Arabia*, vol 24. Karger, Basel; pp 359-372.

Deeming JC (1996) The Calliphoridae (Diptera: Cyclorrhapha) of Oman. In: Krupp F (ed) *Fauna of Arabia*, vol 15. Karger, Basel; pp 264-279.

Derwesh AI (1965) A preliminary list of identified insects and some arachnids of Iraq. *Bulletin, Ministry of Agriculture, Directorate General of Agricultural Research and Projects, Baghdad* 121: 1-123.

Diab FM, Al-Khalifa MS, Al-Asgah NA, Hussein HS and Khalil GA (2006) Ticks (Acari: Argasidae, Ixodidae) infesting livestock in Saudi Arabia. In: Krupp F (ed) *Fauna of Arabia*, vol 22. Karger, Basel; pp 233-242.

Diaz-Aranda LM, Martin-Vega D, Baz A and Cifrian B (2018) Larval identification key to necrophagous Coleoptera of medico-legal importance in the western Palaearctic. *International Journal of Legal Medicine* 132: 1795-1804.

Disney RHL (2006) Scuttle flies (Diptera: Phoridae). Part I: All genera except *Megaselia*. In: Krupp F (ed) *Fauna of Arabia*, vol 22. Karger, Basel; pp 473-521.

Disney RHL (2009) Scuttle flies (Diptera: Phoridae) Part II: the genus *Megaselia*. In: Krupp F (ed) *Fauna of Arabia*, vol 24. Karger, Basel; pp 249-357.

El-Hawagry MS and El-Azab SA (2019) Catalog of the Calliphoridae, Rhiniidae, and Sarcophagidae of Egypt (Diptera: Oestroidea). *Egyptian Journal of Biological Pest Control* 29: e15.

Español F (1979) Coleoptera: Fam. Anobiidae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 1. Karger, Basel; pp 251-256.

Español F (1981) Coleoptera: Fam. Anobiidae (Part 2). In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 3. Karger, Basel; p 266.

Galal LAA, Abd-El-Hameed SY, Attia RAH and Uonis DA (2009) An initial study on arthropod succession on exposed human tissues in Assuit, Egypt. *Mansoura Journal of Forensic Medicine and Clinical Toxicology* 17: 55–74.

Gatrad A (1994) Muslim customs surrounding death, bereavement, post-mortem examinations and organ transplants. *British Medical Journal* 309: 521-523.

GilArriortua M, Saloña Bordas MI, Cainé LM, Pinheiro F and de Pancorbo MM (2013) Cytochrome b as a useful tool for the identification of blow flies of forensic interest (Diptera, Calliphoridae). *Forensic Science International* 228: 132–136.

Gunn A (2019) *Essential Forensic Biology*. 3rd edn. Wiley-Blackwell, Chichester.

Haddadi R, Alajmi R and Abdel-Gaber R (2019) A comparative study of insect succession on rabbit carrion in three different microhabitats. *Journal of Medical Entomology* 56: 671-680.

Haghi FM, Akbarzadeh K, Eslamifar M, Yazdani-Charati J, Movahedi M and Akbari Mohammadi K (2017) Prevalence of the medically important flies at Sari

township, Mazandaran Province. *Journal of Entomology and Zoology Studies** 5: 1344–1347.

Hani M, Thieven U and Perotti MA (2018) Soil bulb mites as trace evidence for the location of buried money. *Forensic Science International* 292: e25-e30.

Háva J (2011) Contribution to the Dermestidae (Coleoptera) from the Arabian Peninsula 1. *Latvijas Entomologs* 50: 5-8.

Háva J (2015) *Dermestidae (Coleoptera)*. Brill, Leiden.

Hegazi EM, Shaaban MA and Sabry E (1991) Carrion insects of the Egyptian western desert. *Journal of Medical Entomology* 28: 734-739.

Heinertz R (1979) Coleoptera: Fam. Carabidae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 1. Karger, Basel; p 140.

Hoogstraal H, Wassef HY and Büttiker W (1981) Fam. Argasidae, Ixodidae. In: Wittmer W and Büttiiker W (eds) *Fauna of Saudi Arabia*, vol 3. Karger, Basel; pp 25-110.

Hoogstraal H (1982) Fam. Ixodidae (Subgenera). In: Wittmer W and Büttiiker W (eds) *Fauna of Saudi Arabia*, vol 4. Karger, Basel; p 22.

Hoogstraal H and Bafort JM (1982) *Ornithodoros (Alectorobius) muesebecki* (Acarina: Fam. Argasidae), parasitizing marine birds in the Arabian Gulf, Saudi Arabia. In: Wittmer W and Büttiiker W (eds) *Fauna of Saudi Arabia*, vol 4. Karger, Basel; pp 23-28.

Hoogstraal H, Büttiker W and Wassef HY (1983a) *Hyalomma (Hyalommina) arabica* (Fam. Ixodidae), a parasite of goats and sheep in Saudi Arabia. In: Wittmer W and Büttiiker W (eds) *Fauna of Saudi Arabia*, vol 5. Karger, Basel; pp 117-120.

Hoogstraal H, Büttiker W and Wassef HY (1983b) *Argas (Persicargas) streptopelia* (Fam. Argasidae), a parasite of doves in Saudi Arabia. In: Wittmer W and Büttiiker W (eds) *Fauna of Saudi Arabia*, vol 5. Karger, Basel; pp 109-116.

Hoogstraal H (1985) Iconography for *Boophilus* and *Hyalomma*: Species identification (Fam. Ixodidae). In: Büttiker W and Krupp F (eds) *Fauna of Saudi Arabia*, vol 6. Karger, Basel; pp 170-174.

Hoogstraal H, Wassef HY, Diab FM, Al-Asgah NA and Al-Khalifa MS (1985) *Ornithodoros (Alveonasus) lahorensis* (Fam. Argasidae) in Saudi Arabia: Biological, veterinary, and medical implications. In: Büttiker W and Krupp F (eds) *Fauna of Saudi Arabia*, vol 6. Karger, Basel; pp 165-169.

Huchet J-B (1996) L'Archéoentomologie funéraire: une approche originale dans l'interprétation des sépultures [Funereal archeoentomology: An original

approach to the interpretation of burials]. *Bulletins et Memoires de la Societe d'Anthropologie de Paris* 8: 299-311.

Ibrahim AA, Galal FH, Seufi AM and Elhefnawy AA (2013) Insect succession associated with corpse decomposition of the guinea pig *Cavia porcellus* in Benha City, Egypt. *Egyptian Academic Journal of Biological Sciences E Medical Entomology and Parasitology* 5: 1-20.

Jelínek J (1988) Coleoptera: Nitidulidae of Saudi Arabia (Part 2). In: Büttiker W and Krupp F (eds) *Fauna of Saudi Arabia*, vol 9. Karger, Basel; pp 42-51.

Kaszab Z (1979) Coleoptera: Fam. Tenebrionidae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 1. Karger, Basel; pp 257-288.

Kaszab Z (1981) Coleoptera: Fam. Tenebrionidae (Part 2). In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 3. Karger, Basel; pp 276-401.

Kaszab Z (1982) Coleoptera: Fam. Tenebrionidae (cont.). In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 4. Karger, Basel; pp 124-243.

Keshavarz iD, Moemenbellah-Fard MD, Zarenezhad M and Gholamzadeh S (2016) First forensic record of blowfly, Calliphora vicina, larvae on an indoor human corpse in winter, south of Iran. *International Journal of Forensic Science and Pathology** 4: 218-220.

Keshavarzi D, Fereidooni M, Assareh M and Nasiri Z (2015a) A checklist of forensic important flies (Insecta: Diptera) associated with indoor rat carrion in Iran. *Journal of Entomology and Zoology Studies** 3: 140-142.

Keshavarzi D, Fereidooni M, Moemenbellah-Fard MD, Nasiri Z, Soltani Z, Dabaghmanesh T and Montazeri M (2015b) Preliminary data on life cycle of *Creophilus maxillosus* Linnaeus (Coleoptera: Staphylinidae) and new report of this species on a human corpse, South of Iran. *International Journal of Forensic Science and Pathology** 3: 144-147.

Keshavarzi D, Moemenbellah-Fard MD, Fereidooni M and Montazeri M (2015c) First report of *Dermestes frischii* Kugelann (Coleoptera: Dermestidae) on a human corpse, south of Iran. *International Journal of Forensic Science and Pathology** 3: 113-115.

Kharoshah MAA, Zaki MK, Galeb SS, Moulana AAR and Elsebaay EA (2011) Origin and development of forensic medicine in Egypt. *Journal of Forensic and Legal Medicine* 18: 10-13.

Klintschar M, Al-Hammadi N, Lux T and Reichenpfader B (1998) Genetic variation at the short tandem repeat loci HumvWA, HumFXIIIB, and HumFES/FPS in the Egyptian and Yemenian populations. *Journal of Forensic Sciences* 43: 850-853.

Kryzhanovskij O (1979) Coleoptera: Fam. Histeridae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 1. Karger, Basel; pp 184-192.

Kwieton E (1981) Coleoptera: Fam. Tenebrionidae, Tribe Pimeliini. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 3. Karger, Basel; pp 402-407.

Li R (2015) *Forensic Biology*. 2nd edn. CRC, Boca Raton.

Madadin M (2015) Forensic autopsy practice in the Middle East and its comparison with the West. *Journal of Forensic and Legal Medicine* 36: 52-53.

Madadin M, Molah R and Cordner S (2017) Autopsy histopathology where the prosector is not a histopathologist: a proposal. *Forensic Science, Medicine, and Pathology* 13: 383-387.

Mandl K (1980) Coleoptera: Fam. Carabidae, Subf. Callistinae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 2. Karger, Basel; pp 95-101.

Mandl K (1986) Coleoptera: Fam. Carabidae, Subf. Callistinae. In: Büttiker W and Krupp F (eds) *Fauna of Saudi Arabia*, vol 7. Karger, Basel; pp 95-101.

Marshall TC, Sunnucks P, Spalton JA, Greth A and Pemberton JM (1999) Use of genetic data for conservation management: the case of the Arabian oryx. *Animal Conservation* 2: 269-278.

Mashaly A, Alajmi R, Mustafa AE-Z, Rady A and Alkhedir H (2017) Species abundance and identification of forensically important flies of Saudi Arabia by DNA barcoding. *Journal of Medical Entomology* 54: 837-843.

Mashaly A, Sharaf M, Al-Subeai M, Al-Mekhlafi F, Aldawood A and Anderson G (2018a) Ants (Hymenoptera: Formicidae) attracted to rabbit carcasses in three different habitats. *Sociobiology* 65: 433-440.

Mashaly AM, Al-Ajmi RA and Al-Johani HA (2018b) Molecular Identification of the carrion beetles (Coleoptera) in selected regions of Saudi Arabia. *Journal of Medical Entomology* 55: 1423–1430.

Mashaly AM, Mahmoud A and Ebaid H (2019) Influence of clothing on decomposition and presence of insects on rabbit carcasses. *Journal of Medical Entomology* tjz038, in press.

Mashaly AMA (2016) Entomofaunal succession patterns on burnt and unburnt rabbit carrion. *Journal of Medical Entomology* 53: 296-303.

Mashaly AMA and Al-Mekhlafi FA (2016) Differential Diptera succession patterns on decomposed rabbit carcasses in three different habitats. *Journal of Medical Entomology* 53: 1192-1197.

Mashaly AMA (2017) Carrion beetles succession in three different habitats in Riyadh, Saudi Arabia. *Saudi Journal of Biological Sciences* 24: 430-435.

Mateu J (1979) Coleoptera: Fam. Carabidae, Subfam. Lebiinae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 1. Karger, Basel; pp 147-155.

Mateu J (1986) Lebiinae and Brachininae of Saudi Arabia (Coleoptera: Carabidae). In: Büttiker W and Krupp F (eds) *Fauna of Saudi Arabia*, vol 8. Karger, Basel; pp 198-218.

Meilia PDI, Freeman MD, Herkutanto and Zeegers MP (2018) A review of the diversity in taxonomy, definitions, scope, and roles in forensic medicine: implications for evidence-based practice. *Forensic Science, Medicine, and Pathology* 14: 460-468.

Menier JJ (1986) Coleoptera: Fam. Cleridae of Saudi Arabia (Part 2). In: Büttiker W and Krupp F (eds) *Fauna of Saudi Arabia*, vol 8. Karger, Basel; pp 219-232.

Mgashaly A, Alajmi R, Mustafa AE, Rady A and Alkhedir H (2017) Species abundance and identification of forensically important flies of Saudi Arabia by DNA barcoding. *Journal of Medical Entomology* 54: 837-843.

Mghirbi T, Aissaoui A, Turki E and Chadly A (2004) Forensic medicine in Tunisia. *Forensic Science International* 146: S33-S35.

Moemenbellah-Fard MD, Keshavarzi D, Fereidooni M, Soltani A, Gholamzadeh S, Montazeri M and Soltani Z (2015) First case report of *Calliphora vicina* (Diptera: Calliphoridae) on an outdoor human corpse with an estimation of postmortem interval from Iran. *Journal of Entomolgy and Zoology Studies** 3: 400-402.

Moemenbellah-Fard MD, Keshavarzi D, Fereidooni M and Soltani A (2018) First survey of forensically important insects from human corpses in Shiraz, Iran. *Journal of Forensic and Legal Medicine* 54: 62-68.

Mohammed M and Kharoshah MA (2014) Autopsy in Islam and current practice in Arab Muslim countries. *Journal of Forensic and Legal Medicine* 23: 80-83.

Mroczkowski M (1979) Coleoptera: Fam. Dermestidae, Part I, Faunistic Data. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 1. Karger, Basel; pp 212-214.

Mroczkowski M (1980) Coleoptera: Fam. Dermestidae (Part 2). Descriptions of three new species. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 2. Karger, Basel.

Nagel P (1982) Coleoptera: Fam. Carabidae, Subfam. Paussinae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 4. Karger, Basel; pp 99-107.

Ota M, Katsuyama Y, Mizuki N, Ando H, Furihata K, Ono S, Pivetti Pezzi P, Tabbara KF, Palimeris GD, Nikbin B, Davatchi F, Chams H, Geng Z, Bahram S and Inoko H (1997) Trinucleotide repeat polymorphism within exon 5 of the MICA gene (MHC class I chain-related gene A): Allele frequency data in the nine population groups Japanese, Northern Han, Hui, Uygur, Kazakhstan, Iranian, Saudi Arabian, Greek and Italian. *Tissue Antigens* 49: 448-454.

Oueidat D, Hussein AH, Assi BT, Daouk H and Jurjus AR (2013) *Forensic medicine in Lebanon: An update*. In: Gulmen MK (ed) 22nd Congress of the International Academy of Legal Medicine (IALM), Istanbul, Turkey, 2012. pp 319-322.

Paulis MG and Faheem AL (2016) Homicidal snake bite in children. *Journal of Forensic Sciences* 61: 559-561.

Perotti MA and Braig HR (2019) Acarology in crimino-legal investigations: the human acarofauna during life and death. In: Byrd JH and Tomberlin JK (eds) *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. 3rd edn. Taylor & Francis, Boca Raton.

Puthz V (1980) Coleoptera: Fam. Staphylinidae, Subfam. Steninae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 2. Karger, Basel; pp 112-113.

Rognes K (2002) Blow flies (Diptera: Calliphoridae) of Israel and adjacent areas, including a new species from Tunisia. *Entomologica Scandinavica* 59 Suppl.: 1-148.

Sajid MI (2016) Autopsy in Islam, considerations for deceased Muslims and their families currently and in the future. *American Journal of Forensic Medicine and Pathology* 37: 29-31.

Salem AM, Adham FK and Picard CJ (2015) Survey of the genetic diversity of forensically important *Chrysomya* (Diptera: Calliphoridae) from Egypt. *Journal of Medical Entomology* 52: 320-328.

Salimi M, Chatrabgoun O, Akbarzadeh K, Oshaghi M, Falahati MH, Rafizadeh S, Yusuf MA and Rassi Y (2018a) Evaluation of insect succession patterns and carcass weight loss for the estimation of postmortem interval. *Journal of Medical Entomology* 55: 1410-1422.

Salimi M, Rassi Y, Ahmadi B, Chatrabgoun O, Jamshidi R and Rafizadeh S (2018b) Effects of morphine on the biomass and development rate of *Chrysomya albiceps* (Diptera: Calliphoridae), a forensically important species. *Tropical Biomedicine* 35: 560-570.

Salimi M, Rassi Y, Chatrabgoun O, Kamali A, Oshaghi MA, Shiri-Ghaleh V, Moradi M, Rafizadeh S, Akbarzadeh K and Parkhideh SZ (2018c) Toxicological analysis of insects on the corpse: A valuable source of information in forensic investigations. *Journal of Arthropod-Borne Diseases*: 219-231.

Salimi M, Rassi Y, Oshaghi M, Chatrabgoun O, Limoee M and Rafizadeh S (2018d) Temperature requirements for the growth of immature stages of blowflies species, *Chrysomya albiceps* and *Calliphora vicina*, (Diptera:Calliphoridae) under laboratory conditions. *Egyptian Journal of Forensic Sciences* 8: e28.

Samšiňák K (1979) Acari from *Musca domestica*. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 1. Karger, Basel; pp 69-74.

Sawaby RF, Hamouly HEL and Abo-El Ela RH (2016) Taxonomic study of the main families of Egyptian Coleoptera with forensic importance. *Life Science Journal** 13: 39-53.

Schawaller W (1981) Coleoptera: Fam. Silphidae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 3. Karger, Basel; pp 231-233.

Scholtz CH (1980) Coleoptera: Scarabaeoidea: Fam.Trogidae (Genus *Trox*). In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 2. Karger, Basel; pp 137-140.

Setyaningrum H and Al Dhafer HM (2014) The Calliphoridae, the blow flies (Diptera: Oestroidea) of Kingdom of Saudi Arabia. *Egyptian Academic Journal of Biological Sciences. A, Entomology* 7: 49–139.

Shaalan EA, El-Moaty ZA, Abdelsalam S and Anderson GS (2017) A preliminary study of insect succession in Al-Ahsaa oasis, in the eastern region of the Kingdom of Saudi Arabia. *Journal of Forensic Sciences* 62: 239-243.

Shaumar N and Mohammed S (1983) Keys for identification of species of the Family Sarcophagidae (Diptera) in Egypt. *Bulletin de la Société Entomologique d'Égypte* 64: 121-135.

Shaumar NF, Mohamed SK and Mohamed SA (1989) Keys for identification of species of family Calliphoridae (Diptera) in Egypt. *Journal of the Egyptian Society of Parasitology* 19: 669-681.

Shayya S, Debruyne R, Nel A and Azar D (2018a) Forensically relevant blow flies in Lebanon survey and identification using molecular markers (Diptera: Calliphoridae). *Journal of Medical Entomology* 55: 1113-1123.

Shayya S, Degallier N, Nel A, Azar D and Lackner T (2018b) Contribution to the knowledge of *Saprinus* Erichson, 1834 of forensic relevance from Lebanon (Coleoptera, Histeridae). *Zookeys* 738: 117-152.

Shayya S, Garrouste R, Nel A and Azar D (2018c) The community succession of arthropods on a pig carcass in Lebanon: different taxonomic level approaches with faunistic notes. *Annales de la Société Entomologique de France* 54: 417-433.

Shiravi AH, Mostafavi R, Akbarzadeh K and Oshaghi MA (2011) Shiravi AH, Mostafavi R, Akbarzadeh K, Oshaghi MA (2011) Temperature requirements

of some common forensically important blow and flesh flies (Diptera) under laboratory conditions. Iran J Arthropoda Borne Dis 5(1):54–62. *Iranian Journal of Arthropod-Borne Diseases* 5: 54–62.

Smith S (1951) The history and development of forensic medicine. *British Medical Journal* 4707: 599-607.

Tahir MA, Al Khayat AQ, Al Shamali F, Budowle B and Novick GE (1997) Distribution of HLA-DQA1 alleles in Arab and Pakistani individuals from Dubai, United Arab Emirates. *Forensic Science International* 85: 219-223.

Taleb M, Tail G and Acikgoz HN (2018) First record of Chrysomya megacephala (Fabricius, 1794) (Diptera: Calliphoridae) in Algeria. *Entomological News* 128: 78-86.

Tantawi TI, El-Kady EM, Greenberg B and El-Ghaffar HA (1996) Arthropod succession on exposed rabbit carrion in Alexandria, Egypt. *Journal of Medical Entomology* 33: 566-580.

Touari M, Mesbah M, Dellatolas G and Bensmail B (1993) Association between criminality and psychosis - A retrospective study of 3984 expert psychiatric evaluations. *Revue d'Épidémiologie et de Santé Publique* 41: 218-227.

Ueckermann EA, Van Harten A and Smith Meyer KP (2006) The mites and ticks (Acari) of Yemen: an annotated check-list. In: Krupp F (ed) *Fauna of Arabia*, vol 22. Karger, Basel; pp 243-286.

Winkler JR (1981) Coleoptera: Fam. Cleridae. In: Wittmer W and Büttiker W (eds) *Fauna of Saudi Arabia*, vol 3. Karger, Basel; pp 251-265.

Zeariya MGM, Hammad KM, Fouda MA, Al-Dali AG and Kabadaia MM (2015) Forensic - insect succession and decomposition patterns of dog and rabbit carcasses in different habitats. *Journal of Entomolgy and Zoology Studies** 3: 473-482.

3 The potential of dust and dust mites in forensic trace analyses

3.1 Introduction

A significant part of crime investigation focuses on the crime scene investigation and the evidence obtained from that scene (Fish et al., 2013). Evidence may take many forms and the successful isolation of samples of reliable and valid sources of evidence remains an important feature of contemporary forensic analysis (Evans and French, 2009; White, 2016; Ramirez and Parish-Fisher, 2017). Although contemporary analysis often revolves around the identification of DNA samples and the accuracy of matching these samples with a perpetrator (Bond and Hammond, 2008), both biological and non-biological material may hold clues regarding important aspects of a crime (Fish et al., 2013). Therefore, identifying useful avenues of forensic analysis within a scene can require a wider consideration of the available evidence.

One of the most ubiquitous components of most crime scenes, particularly those based indoors, is dust (Evett, 1993). Dust is not a single substance, a microscopic accumulation of degraded matter derived from many sources (Gardner and Krouskup, 2018). Within dust, there can be biological and non-biological materials, all of which hold the potential to identify how individuals or objects have interacted with others and the environment (Evett, 1993). Dust is not only a static medium, but also serves as a food source for dust mites, which are common in the house (Gardner and Krouskup, 2018). These mites feed on biological matter, including skin particles, within dust and potentially act as reservoirs for biological matter from a crime scene (Frost et al., 2010). Indeed, forensic acarology is an emerging field that relies on the use of mites in the analysis of trace evidence (Braig and Perotti, 2009; Perotti et al., 2009; Dhooria, 2016; Perotti and Braig, 2019).

Although dust and dust mites are commonplace in crime scenes and have the potential to hold clues regarding the details of the crime and the guilty parties, they are often overlooked resources in forensic trace analysis. The aim of this paper is to provide an overview of the contemporary literature on the potential of dust and dust mites as useful forensic tools during trace analyses.

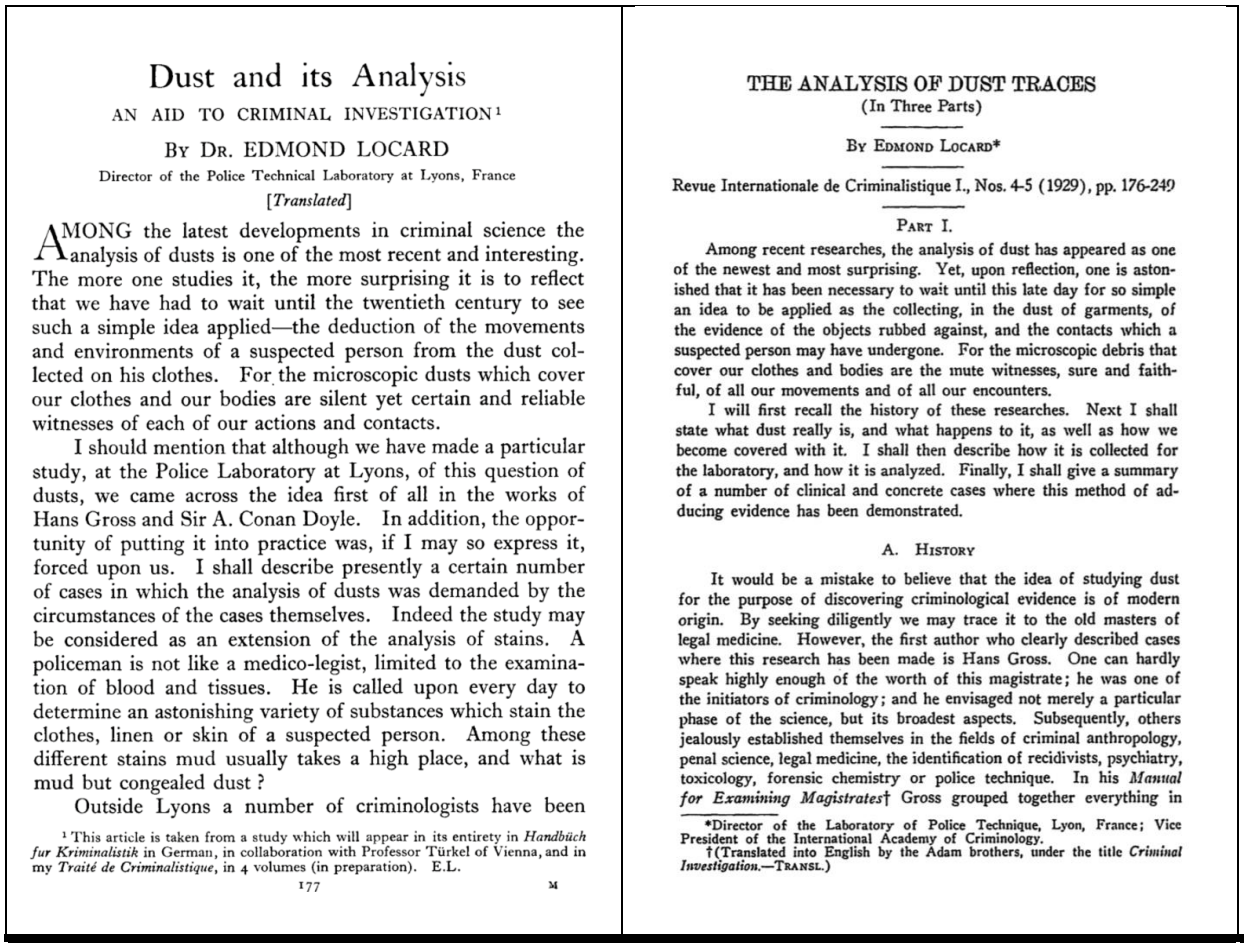
3.2 Dust composition and implications

The recognition that dust could be considered a practically useful avenue for forensic investigation emerged in the early Twentieth Century. Edmond Locard in Lyons France, who built the very first police laboratory, published several papers on the use of dust in forensic trace analysis, generally espousing the virtues and potential of dust as a forensic substance, while demonstrating the deductive capabilities of dust analysis (Locard, 1928). Indeed, dust is generated as a consequence of living and contains biological matter, including skin cells, hair and other degraded tissue, as well as particles associated with clothing and the wider environment (Locard, 1928). As humans spend the majority of their time indoors (sleeping in bed) compared to other locations, dust within this environment may be characteristically and uniquely representative of that individual's lifestyle and habits (Horswell and Fowler, 2003).

Dust is a ubiquitous substance and is highly heterogeneous depending on the substances comprising dust and the local environment (Burney, 2013). Locard considered dust a microcosm of the local environment, comprising microscopic evidence of the interactions between an object and its environment. Dust not only held an important quantitative aspect due to its ubiquity, but also an important qualitative aspect of reflecting its environment and original source. Dust could be thought of as an accumulation of microscopic debris that traced interactions of an object with other people or things, potentially representing a broad range of exposures. Although dust was essentially a pulverised form of these objects and environments, it could be traced back to these origins successfully, a key aspect of the potential forensic yield of dust (Locard, 1928, 1930), Figure 3.1.

Building on the elemental principles of crime scene investigation proposed by Hans (Gross) Groß (1907), Locard attributed importance to the collection of dust, as well as the spatial distribution of dust samples and layering of dust on an object (Locard, 1930). Rigorous methodological approaches to dust sampling, sample labelling and crime scene treatment were characteristic of key changes emerging in early Twentieth Century crime scene management. Consequently, the sampling and analysis of dust became synonymous with a new methodical approach to

investigations, where the integrity of the crime scene was valued (Burney, 2013). While Locard published a review of 22 cases where dust analysis proved beneficial in apprehending criminals, he readily admitted that the fictitious detective series of the time (including Sherlock Holmes) heavily influenced his work and blurred the boundaries and expectations of practical dust analysis and fantasy (Berg, 1970).



Dust and its Analysis

AN AID TO CRIMINAL INVESTIGATION[1]

BY DR. EDMOND LOCARD

Director of the Police Technical Laboratory at Lyons, France

[*Translated*]

AMONG the latest developments in criminal science the analysis of dusts is one of the most recent and interesting. The more one studies it, the more surprising it is to reflect that we have had to wait until the twentieth century to see such a simple idea applied—the deduction of the movements and environments of a suspected person from the dust collected on his clothes. For the microscopic dusts which cover our clothes and our bodies are silent yet certain and reliable witnesses of each of our actions and contacts.

I should mention that although we have made a particular study, at the Police Laboratory at Lyons, of this question of dusts, we came across the idea first of all in the works of Hans Gross and Sir A. Conan Doyle. In addition, the opportunity of putting it into practice was, if I may so express it, forced upon us. I shall describe presently a certain number of cases in which the analysis of dusts was demanded by the circumstances of the cases themselves. Indeed the study may be considered as an extension of the analysis of stains. A policeman is not like a medico-legist, limited to the examination of blood and tissues. He is called upon every day to determine an astonishing variety of substances which stain the clothes, linen or skin of a suspected person. Among these different stains mud usually takes a high place, and what is mud but congealed dust ?

Outside Lyons a number of criminologists have been

[1] This article is taken from a study which will appear in its entirety in *Handbüch fur Kriminalistik* in German, in collaboration with Professor Türkel of Vienna, and in my *Traité de Criminalistique*, in 4 volumes (in preparation). E.L.

177 M

THE ANALYSIS OF DUST TRACES

(In Three Parts)

BY EDMOND LOCARD*

Revue Internationale de Criminalistique I., Nos. 4-5 (1929), pp. 176-249

PART I.

Among recent researches, the analysis of dust has appeared as one of the newest and most surprising. Yet, upon reflection, one is astonished that it has been necessary to wait until this late day for so simple an idea to be applied as the collecting, in the dust of garments, of the evidence of the objects rubbed against, and the contacts which a suspected person may have undergone. For the microscopic debris that cover our clothes and bodies are the mute witnesses, sure and faithful, of all our movements and of all our encounters.

I will first recall the history of these researches. Next I shall state what dust really is, and what happens to it, as well as how we become covered with it. I shall then describe how it is collected for the laboratory, and how it is analyzed. Finally, I shall give a summary of a number of clinical and concrete cases where this method of adducing evidence has been demonstrated.

A. HISTORY

It would be a mistake to believe that the idea of studying dust for the purpose of discovering criminological evidence is of modern origin. By seeking diligently we may trace it to the old masters of legal medicine. However, the first author who clearly described cases where this research has been made is Hans Gross. One can hardly speak highly enough of the worth of this magistrate; he was one of the initiators of criminology; and he envisaged not merely a particular phase of the science, but its broadest aspects. Subsequently, others jealously established themselves in the fields of criminal anthropology, penal science, legal medicine, the identification of recidivists, psychiatry, toxicology, forensic chemistry or police technique. In his *Manual for Examining Magistrates*† Gross grouped together everything in

*Director of the Laboratory of Police Technique, Lyon, France; Vice President of the International Academy of Criminology.

†(Translated into English by the Adam brothers, under the title *Criminal Investigation*.—TRANSL.)

Figure 3.1 – Title pages of the English translation of Locard's papers on dust from 1928 and 1929 (Locard, 1928, 1930). These papers form part of the work that established Locard's principle of transfer of trace evidence and are the very first that define dust as forensic trace evidence (Locard, 1931-1936). These two English translations were both published in the first volumes of a European and an American forensic journal: *The Police Journal* and *American Journal of Police Science*.

During the Twentieth Century, techniques developed rapidly, and forensic analysis became more nuanced. While dust analysis played a role in some criminal investigations, it was an appreciably small role and often limited to very specific cases (Burney, 2013). Indeed, the analysis of blood, hair and fibres was more

typically considered to be valuable in identifying criminals and establishing other facts surrounding a crime, often with a greater degree of precision than dust analysis. However, the principles of dust analysis remain valid and representative of the rigour and methods used in contemporary forensic analysis.

3.3 Dust mites

In addition to microscopic particles of matter from the environment comprising forensically sample dust, as well as blood, hair and fibre analysis, the Twentieth Century saw a rise in the use of insects as means of establishing information in a forensic case (Smith, 1986; Catts and Haskell, 1990; Goff, 2000; Erzinçlioğlu, 2002; Greenberg and Kunich, 2002; Amendt et al., 2010; Gennard, 2012; Wyss and Cherix, 2013; Charabidze and Gosselin, 2014; Rivers and Dahlem, 2014; Byrd and Tomberlin, 2019). Arthropods have an established role in estimating time of decomposition of bodies as part of forensic evaluation (Gennard, 2012). However, the forensic potential of insects has wider implications in other crimes and forensic contexts. Of particular interest, and relevance to dust analysis, is the use of dust mites in forensic capacities, Figure 3.2.

Dust mites are found in household dust and are a medically important group of mites, often associated with allergic reactions (Calderon et al., 2015). Over 150 types of mites have been identified in the household, while three species are particularly common and consistently seen in the house: *Dermatophagoides pteronyssinus, D. farinae* and *Euroglyphus maynei* (Acariformes: Pyroglyphidae) (Blythe et al., 1974; Crowther et al., 2000). Mites form the most prevalent invertebrate within human houses, although official estimates of all species or orders are difficult due to the wide adaptability of mites and the diversity in the microenvironments in human houses (Frost et al., 2009). What is known is that mites prefer to establish colonies only when humans are present within the house, with an increased level of mites proportional to the number of human residents and the size of the house (Frost et al., 2010). Characterisation of mites living on clothing, mattresses and other regions in close contact with human bodies has been

performed to a high degree and the majority of these mites are preferential consumers of human skin (Calderon et al., 2015).

Based on the potential to characterise mites in this way, the forensic potential of dust mites is an area of interest (Frost et al., 2009). Indeed, mites that consume human skin may concentrate DNA within their digestive systems, yielding advantages over fibre analysis, which does not allow identification of DNA samples (Grieve and Wiggins, 2001). Further appreciation of the dust mite digestive process and potential to hold DNA from human samples is needed to extend the potential value of trace analysis with these organisms. Dust mites consume the epithelial layer of skin following shedding. The digestive process is initiated through mechanical breaking of skin cell fragments in the foregut, followed by endogenous enzyme degradation (Cakan et al., 2015). Digestion of skin cells is incomplete within the digestive tract and both undigested and digested matter are coated in membrane and transferred to the rectum (Hallas, 1991). This membranous digestive product is excreted in the faeces but contains a sufficient quantity of enzymes to continue skin cell breakdown and digestion and mites may consume their faeces to obtain further nutritional content as a consequence (Hallas, 1991). The process of digestion and faecal deposition in dust mites is well described and temporal control of this process holds a potential to estimate DNA content in digestive tracts based on time of skin cell deposition in the local environment (Cakan et al., 2015).

Figure 3.2 – Aggregate of house dust mites (*Dermatophagoides pteronyssinus*) on fabric. Picture Gilles San Martin.

Dust mites and other insects have been used to estimate time of death and the location (or re-location) of bodies during forensic evaluation (Perotti, 2009; Szelecz et al., 2018; Perotti and Braig, 2019), leading to the potential use of mites in more complex forensic analysis, potentially complementing dust analysis. Based on the background of dust and dust mite use in forensic science, there are clear areas where the potential of these forensic reservoirs can be exploited. The following sections consider the evidence base for studies employing dust analysis or dust mite analysis techniques in contemporary literature, followed by a consideration of the future opportunities and challenges of using dust and dust mites in forensic trace analysis.

3.4 Evidence for dust use in contemporary trace analysis

Although modern forensic techniques have focused on improving analytical methods and processes, there remain a number of limitations to the current techniques and implementation of techniques in practice (Stoney and Stoney, 2015). Accordingly, the use of novel approaches and the potential to improve the analysis

of existing approaches have both been proposed as potential factors to enhance current forensic trace analysis (Stoney and Stoney, 2015). Indeed, one of the challenges in contemporary forensic analysis is the need for increasingly accurate analysis of trace materials, while sample sizes become smaller (Butler, 2015). This is particularly true of DNA analysis techniques, where there is an expectation of accuracy in DNA profiling based on minute DNA samples, termed low template DNA (Alfonse et al., 2017). Low template DNA is often found in contexts where an individual may have touched an object (leaving behind only small traces of DNA) or in the context of dust deposition and accumulation within the scene (Szkuta et al., 2015). Therefore, exploiting the value of dust as a reservoir for low template DNA represents an opportunity to increase the sensitivity of DNA analysis for forensic purposes.

Dust analysis is a complex process to identify within the contemporary literature, as dust may be defined in different ways and subtypes of dust or deposited particulates may be focused on in texts. For instance, dust analysis may be considered relevant when determining gunshot residue concentrations or presence in an environment (with or without dust comprising predominantly human material) (Siegel and Mirakovits, 2015), while specific types of dust transferred to other environments have been widely described in studies of specific agents, e.g. coal dust or brick dust (Locard, 1928). Therefore, the contemporary use of dust analysis is widespread and has in many ways become a routine aspect of forensic investigation.

A specific focus on household dust and dust with a significant proportion of human skin is less common within the published literature. Dust may be found in thin layers on household objects, or contained within garments and textiles (Barberán et al., 2015). In addition, this dust is often also aerosolised, with significant quantities of dust within a given room and easy transfer of dust from one material to another (Prussin and Marr, 2015; Craine et al., 2017). Furthermore, dust may accumulate in small aggregates ('dust bunnies'), containing dust, fibres, hair and other trace materials, which are more persistent and reflect a larger forensic potential (Farash, 2015). Strategies to analyse dust particles and to extract valuable

forensic information are often based on blind swabbing techniques, but these are often inadequate and produce artificial mixing of discrete elements in the scene. Furthermore, analytical techniques that rely on a sufficient quantity of one material for positive identification are insufficient when considering dust particle analysis in many routine crime scene contexts (Brown, 2016). Unless, as seen in the works of Locard (1930), there is a substantial quantity of dust that may be related to a specific environment, it is challenging to deduce dust transfer from one area to another. The analysis of DNA within dust represents an area where dust analysis may become increasingly useful, as relative quantities of DNA needed for analysis are small (Bond and Hammond, 2008).

DNA was unknown to Locard and his contemporaries and identification of DNA as part of forensic investigation occurred decades after the initial analyses of dust particles revolutionised trace analysis. Although the potential of DNA analysis within dust could not be exploited by Locard at the time, more recently it has been asserted that dust may contain traces of DNA that can be used in a forensic capacity (Wickenheiser, 2002). Indeed, as individuals touch objects or move through an environment, it is highly likely that skin cells and particulate matter will be transferred, including cells containing trace samples of DNA from the individual. Therefore, in principle crime scenes may contain DNA from the perpetrator within seemingly innocuous or non-descript dust (Wickenheiser, 2002). The potential to combine an analysis of dust particles and DNA identification suggests that dust may once again be realised as a forensic medium capable of enhancing contemporary techniques in the laboratory.

Some of the key challenges with extracting DNA from dust particles relates to the technical challenge of how one physically isolates relevant DNA from dust samples, as well as the potential for multiple sources of contamination depending on the nature of the crime scene (Szkuta et al., 2015). Dust is composed of a range of particles and degraded matter and the relative proportion of dust that contains DNA may be particularly low, further complicating the extraction or identification of DNA within a sample. The following section considers one possible mechanism

through which DNA may be obtained in dust, using a more specific method: dust mite analysis.

3.5 Evidence for dust mite use in contemporary trace analysis

Although it is recognised that dust mites have the potential for crime scene investigation, few studies have attempted to exploit this forensic potential. The majority of texts on forensic acarology or mite use in forensic contexts tend to focus on the value of these mites in establishing age of decay or other factors associated with an identified corpse (e.g. (OConnor, 2009; Turner, 2009; OConnor et al., 2015)). However, this excludes the potential role of dust mites as reservoirs of human DNA in active cases. Environmental DNA has been detected in household dust and in small insects in the environment or house, including in mites (Barnes and Turner, 2016). Therefore, dust mites may harbour human DNA of relevance to crime scene investigation. As the mites feed on human skin cells, there is a possibility that mites are a specifically concentrated source of human DNA, due to their digestive peculiarities, while also providing timescales relating to DNA deposition and/or ingestion (Valerio et al., 2005).

There is evidence to suggest that human DNA extraction from mites is possible, although not based on dust mites. Szalanski et al. (2006b) performed an analysis of blood-engorged bed bugs (*Cimex lectularius*) to establish the potential for DNA extraction. The study involved a single human host exposed to the bed bugs within a given time period and in a controlled environment. The bed bugs were then preserved by drying and storing in 70% ethanol or freezing. DNA extraction was completed and polymerase chain reaction was used to amplify DNA present using microsatellite markers, mitochondrial DNA hypervariable region markers, and insect DNA markers. Amplification of these markers was successful suggesting that DNA extracted from these insects could be sufficient in quality and quantity to permit DNA profiling. A follow-up study suggested that DNA extraction could be viable for up to 60 days post-feed in bed bugs (Szalanski et al., 2006a). Importantly, however, the analysis of blood-eating (haematophagous) insects differs from that in dust mites and there is no guarantee that DNA samples would be as robust in dust

mite populations. Therefore, data from other insect or mite populations may not be applicable to the potential of dust mites in forensic trace analysis.

Despite the potential value of dust mites in the trace analysis, only one study was identified during a literature search that focused on the use of dust mites in this way. Cakan et al. (2015) used a modified vacuum cleaner to collect dust samples from 27 houses and mites were isolated from the dust samples. Blood samples were also obtained from the residents of the dwellings where dust samples were obtained (n=40) for comparison of DNA found in dust mites. DNA isolation from blood samples was performed using standard techniques, using proprietary DNA extraction kits. The same kits were used in dust mite samples and then polymerase chain reaction was performed to amplify DNA obtained from dust mites. Amplification was based on nine microsatellite loci present in DNA samples, followed by capillary electrophoresis and chromatography of the samples.

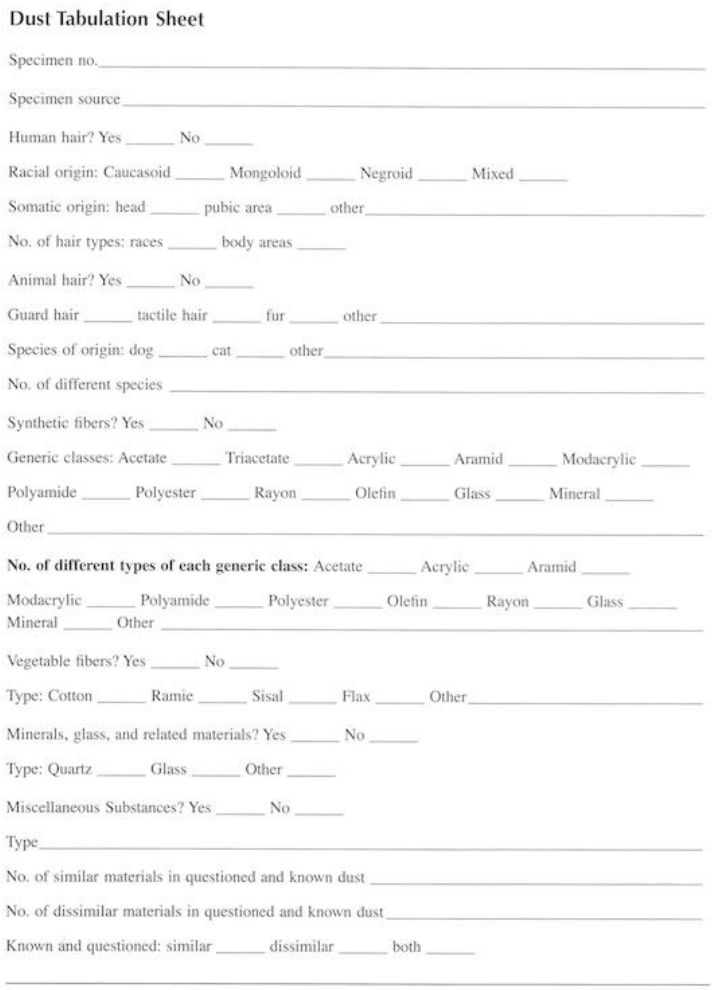
The study found that DNA was detected in 10 % of the isolated house dust mites, with DNA found in 26 out of the 27 dwellings. In total, 1,740 mite samples, across different species, were evaluated. DNA results showed profiling of DNA from four individuals was possible, with exact matches noted between mite DNA and residents of the house from which the mites were obtained. Therefore, this study highlights the potential for DNA extraction and profiling based on house dust mite samples, showing correlation between house dust mite DNA and the DNA of household residents. Although the study only successfully demonstrated DNA profiling in 10 % of household members, the study provides an important proof of principle for this technique, which may be highly accurate in instances of mite contamination of a crime scene. Comparison of dust mite DNA samples with those of potential victims or perpetrators in a specific crime may therefore have some putative value, without the need for additionally expensive or technically complex techniques compared to standard DNA profiling (Cakan et al., 2015).

In addition to the use of human DNA contained within dust mites, characterisation of dust mite subspecies and populations may also form a basis for trace analysis (Thomas, 2010). Mite populations are heterogeneous and have been characterised according to specific environments or microenvironments, based on

desirable living characteristics and access to food (Thomas, 2010). Human clothing and mattress dust mite populations are distinct from those found in kitchens and other rooms of the house. Therefore, molecular characterisation of dust mites from a crime scene has the potential to identify contaminant sources and mite populations consistent with specific points or origin (Frost et al., 2010). However, the author was not able to identify any studies where mite molecular phenotyping was employed in a forensic capacity, suggesting that this remains a theoretical use for dust mite trace analysis. Therefore, this issue will be considered in the following section, which focuses on the future potential, opportunities and challenges of using dust or dust mites in forensic trace analysis.

3.6 Future perspectives, potentials and challenges

The use of dust or dust mites in forensic trace analysis has received relatively little attention in the research literature, but both avenues hold promise in addressing unmet needs in the forensic field and in providing novel approaches to crime scene investigation. The examples of dust and dust mite analysis described in the literature reflect several key opportunities and challenges to the use of these media. This section will consider how these areas may be advanced and the role of dust and dust mites in forensic trace analysis.



Dust Tabulation Sheet

Specimen no. _____

Specimen source _____

Human hair? Yes _____ No _____

Racial origin: Caucasoid _____ Mongoloid _____ Negroid _____ Mixed _____

Somatic origin: head _____ pubic area _____ other _____

No. of hair types: races _____ body areas _____

Animal hair? Yes _____ No _____

Guard hair _____ tactile hair _____ fur _____ other _____

Species of origin: dog _____ cat _____ other _____

No. of different species _____

Synthetic fibers? Yes _____ No _____

Generic classes: Acetate _____ Triacetate _____ Acrylic _____ Aramid _____ Modacrylic _____

Polyamide _____ Polyester _____ Rayon _____ Olefin _____ Glass _____ Mineral _____

Other _____

No. of different types of each generic class: Acetate _____ Acrylic _____ Aramid _____

Modacrylic _____ Polyamide _____ Polyester _____ Olefin _____ Rayon _____ Glass _____
Mineral _____ Other _____

Vegetable fibers? Yes _____ No _____

Type: Cotton _____ Ramie _____ Sisal _____ Flax _____ Other _____

Minerals, glass, and related materials? Yes _____ No _____

Type: Quartz _____ Glass _____ Other _____

Miscellaneous Substances? Yes _____ No _____

Type _____

No. of similar materials in questioned and known dust _____

No. of dissimilar materials in questioned and known dust _____

Known and questioned: similar _____ dissimilar _____ both _____

Figure 3.3 – The long way to go: no mites in dust. Dust Tabulation Sheet, page 183, in Petraco and Kubic (2004).

Combining dust analysis or dust mite analysis with DNA/molecular analysis techniques appears to be a promising strategy for the future use of these media in forensic contexts. DNA identification in particular is an established aspect of contemporary forensics, with microsatellite identification forming an important aspect of DNA profiling and criminal investigation (Butler, 2005). Microsatellites are short repetitive sequences of DNA, typically ranging from 1-10 base pairs in length and repeating 10-50 times (Urquhart et al., 1994). These are also termed short tandem repeats (STRs) and occur frequently within the human genome (Butler, 2015). The vast majority of STRs are found in non-coding regions of the genome, where they have no role in protein encoding or any further biological functions (Elkins, 2012). Within non-coding regions, mutations within STRs can accumulate over time, as these regions are not under any selective pressure during DNA

replication and organism reproduction (Kayser, 2015). Therefore, STRs in non-coding regions can accumulate unique mutations in specific individuals over generations, leading to the potential for DNA profiling (fingerprinting) (Kayser, 2015).

The complexity of using dust for trace analysis in contemporary forensics relates to the potential for limited DNA within specific dust samples (depending on the nature of dust deposition and the local environment) as well as the converse, an abundance of DNA and sample contamination (Poy and van Oorschot, 2006). Indeed, Kester et al. (2010) analysed dust samples using swabs from an indoor environment and found that 97 % of samples contained human DNA. Of these samples, 61 % contained sufficient DNA to yield allele distributions of varying degrees of complexity based on STR analysis. This study has several implications for the use of dust in forensic analysis: firstly, that dust is a good source of human DNA within an indoor environment (which may be negative where dust is considered a crime scene contaminant), secondly, that multiple sources of DNA may be contained within samples from a relatively small area, requiring effective techniques to exclude inhabitants from a defined region and sorting of DNA genotypes (Toothman et al., 2008). Rigorous implementation of dust collection protocols and sample collection in general may overcome some limitations, while more effective genotyping approaches and refinement techniques would be needed to assess the DNA profiles contained within dust.

There is some evidence that these obstacles may be overcome in contemporary analytical settings. For instance, Craine et al. (2017) recently showed that house dust analysis could be used to characterise environmental plant DNA with a high degree of accuracy, including characterisation of indoor and outdoor plants and potential allergic plant materials. However, this study did not analyse human DNA and was not applied to the forensic criminal context. An analysis of dust from the Turin shroud, supposedly used as the burial cloth for Jesus Christ, was also used to identify plant DNA and mitochondrial DNA from humans, including identification of specific ethnic groups based on DNA haplotyping (Barcaccia et al., 2015). Therefore, the principle of extracting suitable amounts or DNA from dust

samples appears to be proven in contemporary analyses. However, it should be noted that determination of the precise origin of DNA samples within dust in these studies is an issue of contention, as recent handling or older handling may have resulted in the contamination (Barcaccia et al., 2015). Therefore, more rigor is needed to ensure that DNA profiling based on dust samples can be accurate and contemporary in nature to have a role in legal proceedings.

Farash (2015) reported that the use of an enhanced and refined strategy for dust collection could facilitate adequate DNA sampling from household dust sources, including aggregated dust, leading to trace analysis potential. While this strategy, which involved the use of adhesive microscope slides and specific processes to extract biological materials from within dust conglomerates, can facilitate more accurate and sensitive DNA assessment in a crime scene context, there are challenges remaining to using dust in this way. This includes the difficulty in establishing not only the donor source of DNA, but also the tissue specificity of DNA samples, as this information may be helpful in the context of criminal investigation (Zubakov et al., 2018). The development of tissue-specific messenger RNA markers (Farash, 2015) is one strategy to overcome this limitation and to enhance recovered DNA for profiling purposes.

Another limitation relates to the ability to define the time when DNA was deposited at the scene within dust samples. Ostojic and Wurmbach (2017) recently showed that DNA profiling could be made successfully after 40 days of degradation in samples, based on recreation of household characteristics, including dust levels and contaminants. However, the ability to differentiate between DNA deposited at different time points represents a clear technical challenge, as samples need to be sufficient to allow for DNA analysis and may not allow for stratification or rigour in sample attainment, as recommended by Locard (1930). The use of dust mites in the analysis of DNA may offer temporal advantages to dust evaluation alone, based on the characteristics of dust mite lifespans and digestive processes (Dhooria, 2016).

Many dust mites reproduce sexually and their rate of reproduction and overall life cycle are heavily dependent on environmental factors, particularly humidity and temperature (Colloff, 2009). Outside of their normal range of

humidity and temperature, reproduction and lifespan are severely reduced (Miller, 2018). In optimal conditions, mites may live for 122 days or more, while suboptimal conditions may reduce mite lifespans to 35 days or less (Arlian and Platts-Mills, 2001). Within this lifespan, digestion is a relatively rapid process and therefore recent digested DNA may not be expected to reside within the gastrointestinal tract of mites for long periods of time (Arlian and Morgan, 2003). Hence, dust mites (as well as dust mite faeces) represent a dynamic source of DNA, reflecting recent ingestion, although this may not be directly related to the time of DNA deposition (Arlian and Morgan, 2003). Furthermore, mites are most likely to consume DNA from residents of a house or area, or frequent visitors, rather than a single visitor. This may limit the potential use of dust mites as a resource for identifying criminals or exposures that are transient in nature. However, dust mites persist on clothing and other fabrics, often transported with individuals between environments, suggesting that their utility may be based on the DNA they consume on a regular basis when deposited in new environments (Colloff, 2009).

The principles of Locard's conceptualisation of forensic trace analysis can be applied to dust mites and dust analysis, but clearly with a large degree of complexity. It is apparent that dust and dust mites may contain DNA from individuals and that DNA may be extracted from these sources under optimal conditions. The use of STR/microsatellite analyses, typically four or five base pair sequences, can yield positive DNA profiling results from these sources, indicating that trace DNA persists and transfers along with dust mites and dust during any interaction. However, interpretation of these data and accurate characterisation of the exposure on a quantifiable and temporal basis is an important element of criminal proceedings and may be lacking in current processes and evidence.

Despite the opportunities and novel strategies that may be used to enhance the role of dust analysis and dust mites in forensic trace analysis, there remain several key challenges to the routine use of these techniques in trace analysis. For instance, the risk of contamination of dust samples in crime scenes is high (Fonnelop et al., 2015), particularly with regards to DNA contamination that may occur in a number of ways and within a short period of time. Contamination during evidence

collection or crime scene management may be limited by the use of procedure and protocols, but other types of contamination due to the local environment or level of human traffic in the environment may lead to high levels of uncertainty during analysis (Szkuta et al., 2015). Therefore, application of dust or dust mite trace analysis should be considerate of the local environment and should only be considered where the results may have practical value. Other limitations include the technical challenges in optimising DNA extraction from dust or dust mites and the protocols used to ensure temporal aspects to exposure and DNA deposition/transfer at the scene of the crime.

An obstacle associated with dust mite use in forensic contexts is the capacity to differentiate between mite populations on a molecular level. This is an important point, as mites are ubiquitous in human environments and contamination of crime scenes and evidence with mites from exogenous sources is a possibility in numerous contexts (Frost et al., 2010). As mite populations have been well characterised to the degree that subspecies or orders are most commonly associated with particular environments, molecular phenotyping and markers to differentiate between two human mite populations can assist in identifying contaminating samples (Solarz, 2009). These techniques would need to be developed further, in conjunction with environmental characterisation of mite species, to ensure accuracy in analysis and interpretation of findings.

The Twentieth Century witnessed a massive expansion in the techniques and processes used to establish guilt in criminal procedures, largely due to the pioneering work of forensic investigations. DNA profiling remains one of the most widely used methods to incorporate trace analysis into criminal proceedings and forensic scientists are under increasing pressure to provide positive identification with increasingly small samples of DNA. Accordingly, there is a need for novel strategies to meet the needs of contemporary criminal science.

The use of dust and dust mites for trace analysis and DNA microsatellite-based profiling has been established in principle and remains an overlooked area of forensic investigation. The advantages of these techniques include the capacity to obtain sufficiently large samples of DNA from dust or dust mites compared to other

sources of low template DNA, while the DNA obtained from these samples may be specific to individuals exposed to specific environments, or within a given environment. Dust mites, in particular, provide potential for a high concentration of DNA, due to the selectivity of skin consumption among other particulate matter, and their characteristics, including lifespan, preferred microenvironment and connection to a host, provide intriguing avenues for forensic trace analysis. However, the potential of dust mites or dust in contemporary forensics has yet to be realised.

It is recommended that further studies are conducted to establish the validity and utility of dust and dust mite evaluation in forensic trace analysis. Indeed, there is a paucity of contemporary studies assessing the potential advantages and challenges of these media, while only one contemporary study demonstrates DNA profiling consequent to dust mite analysis. These studies should be performed in accordance with the principles of crime scene investigation and should aim to determine how specific and sensitive analyses based on dust and dust mites can be within the context of criminal evidence proceedings. Furthermore, characterisation of molecular markers allowing for simple separation of dust mite subspecies will be required to exploit the full potential of this approach. Pending these advances, forensic scientists should continue to optimise DNA profiling protocols to ensure sensitivity and specificity of DNA profiling can be maximised, regardless of the source.

3.7 Conclusions

Dust mites have the potential of adding valuable information to forensic trace evidence as long as they can be characterized to a near individual level. If individual dust mites can be ascribed to specific populations, they might attain the same forensic value as currently is hold by human blood traces. The necessary level of molecular resolution can be achieved, like in humans, with the help of microsatellite analysis.

3.8 References

Amendt J, Campobasso CP, Goff ML and Grassberger M (eds) (2010) *Current Concepts in Forensic Entomology*. Springer, Dordrecht.

Arlian LG and Platts-Mills TA (2001) The biology of dust mites and the remediation of mite allergens in allergic disease. *Journal of Allergy and Clinical Immunology* 107: S406-S413.

Arlian LG and Morgan MS (2003) Biology, ecology, and prevalence of dust mites. *Immunology and Allergy Clinics of North America* 23: 443-468.

Barberán A, Dunn RR, Reich BJ, Pacifici K, Laber EB, Menninger HL and Fierer N (2015) The ecology of microscopic life in household dust. *Proceedings of the Royal Society Biological Sciences Series B* 282: e20151139.

Barcaccia G, Galla G, Achilli A, Olivieri A and Torroni A *DNA analysis of dust particles sampled from the Turin Shroud*. In: Fanti G (ed) Workshop of Paduan Scientific Analysis on the Shroud, Padua, Italy, 2015. MATEC Web of Conferences, p e03001.

Barnes MA and Turner CR (2016) The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics* 17: 1-17.

Berg SO (1970) Sherlock Holmes: Father of scientific crime and detection. *Journal of Criminal Law, Criminology and Police Science* 61: 446-452.

Blythe ME, Williams JD and Smith JM (1974) Distribution of pyroglyphid mites in Birmingham with particular reference to *Euroglyphus maynei*. *Clinical and Experimental Allergy* 4: 25-33.

Bond JW and Hammond C (2008) The value of DNA material recovered from crime scenes. *Journal of Forensic Sciences* 53: 797-801.

Braig HR and Perotti MA (2009) Carcasses and mites. *Experimental and Applied Acarology* 49: 45-84.

Brown TA (2016) *Gene Cloning and DNA Analysis: An Introduction*. John Wiley & Sons, New York.

Burney I (2013) Our environment in miniature: Dust and the early twentieth-century forensic imagination. *Representations* 121: 31-59.

Butler JM (2005) *Forensic DNA Typing: Biology, Technology, and genetics of STR Markers*. 2 edn. Elsevier, Amsterdam; pp 660.

Butler JM (2015) The future of forensic DNA analysis. *Philosophical Transactions of the Royal Society of London B Biological Sciences* 370: e20140252.

Byrd JH and Tomberlin JK (eds) (2019) *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. 3rd edn. Taylor & Francis, Boca Raton.

Cakan H, Güven K, Çevik FE, Demirci M and Saribas S (2015) Investigation of human DNA profiles in house dust mites: Implications in forensic acarology. *Romanian Journal of Legal Medicine* 23: 187-192.

Calderon MA, Linneberg A, Kleine-Tebbe J, De Blay F, de Rojas DHF, Virchow JC and Demoly P (2015) Respiratory allergy caused by house dust mites: What do we really know? *Journal of Allergy and Clinical Immunology* 136: 38-48.

Catts EP and Haskell NH (1990) *Entomology and death: A Procedural Guide*. Joyce's Print Shop, Clemons, S.C.

Charabidze D and Gosselin M (eds) (2014) *Insectes, Cadavers ét Scènes de Crime. Principes et applications de l'entomogie médico-légale [Insects, Cadavers and Crime Scenes. Principles and applications of forensic entomogy]*. de boek, Louvain-la-Neuve.

Colloff MJ (2009) *Dust Mites*. Springer, Dordrecht; pp 583.

Craine JM, Barberán A, Lynch RC, Menninger HL, Dunn RR and Fierer N (2017) Molecular analysis of environmental plant DNA in house dust across the United States. *Aerobiologia* 33: 71-86.

Crowther D, Horwood J, Baker N, Thomson D, Pretlove S, Ridley I and Oreszczyn T (2000) *House Dust Mites and the Built Environment: A Literature Review*. University College London, London.

Dhooria MS (2016) Forensic acarology. In: *Fundamentals of Applied Acarology* Springer, Singapore; pp 441-448.

Elkins KM (2012) *Forensic DNA Biology: A Laboratory Manual*. Academic Press, London.

Erzinçlioğlu Z (2002) *Maggots, Murder, and Men: Memories and Reflections of a Forensic Entomologist*. Thomas Dunne Books, New York; pp 256.

Evans C and French JL (2009) *Crime Scene Investigation*. Infobase, Chicago.

Evett IW (1993) Establishing the evidential value of a small quantity of material found at a crime scene. *Science and Justice* 33: 83-86.

Farash K (2015) *Strategies for Enhanced Genetic Analysis of Trace DNA from Touch DNA Evidence and Household Dust*. University of Central Florida, Orlando, FL

Fish JT, Miller LS, Braswell MC and Wallace Jr EW (2013) *Crime Scene Investigation*. Routledge, London.

Fonnelop AE, Egeland T and Gill P (2015) Secondary and subsequent DNA transfer during criminal investigation. *Forensic Science International: Genetics* 17: 155-162.

Frost CL, Braig HR, Amendt J and Perotti MA (2010) Indoor arthropods of forensic importance: Insects associated with indoor decomposition and mites as indoor markers. In: Amendt J, Campobasso CP, Goff ML and Grassberger M (eds) *Current Concepts in Forensic Entomology*. Springer, Dordrecht; pp 93-108.

Gardner RM and Krouskup D (2018) *Practical Crime Scene Processing and Investigation*. 3rd edn. CRC Press.

Gennard D (2012) *Forensic Entomology: An Introduction*. 2nd edn. Wiley-Blackwell, Chichester.

Goff ML (2000) *A Fly for the Prosecution: How Insect Evidence Helps Solve Crimes*. New edited edn. Harvard University Press, Cambridge; pp 234.

Greenberg B and Kunich JC (2002) *Entomology and the Law: Flies as Forensic Indicators*. Cambridge University Press, Cambridge; pp 306.

Grieve MC and Wiggins KG (2001) Fibers under fire: suggestions for improving their use to provide forensic evidence. *Journal of Forensic Sciences* 46: 835-843.

Groß H (1907) *Criminal Investigation: A Practical Handbook for Magistrates, Police Officers and Lawyers* (trans: Collyer Adam J). Specialist Press, London.

Hallas TE (1991) The biology of mites. *Allergy* 46: 6-9.

Horswell J and Fowler C (2003) Associative evidence – the Locard exchange principle. In: Horswell J (ed) *The Practice of Crime Scene Science*. CRC Press, Booca Raton; pp 45-56.

Kayser M (2015) Forensic DNA phenotyping: Predicting human appearance from crime scene material for investigative purposes. *Forensic Science International: Genetics* 18: 33-48.

Kester KM, Toothman MH, Brown BL, Street IV WS and Cruz TD (2010) Recovery of environmental human DNA by insects. *Journal of Forensic Sciences* 55: 1543-1551.

Locard E (1928) Dust and its analysis: An aid to criminal investigation. *Police Journal London* 1: 177-192.

Locard E (1930) The analysis of dust traces. *American Journal of Political Science* 1: 276-286, 401-418, 496-514.

Locard E (1931-1936) *Traité de criminalistique: 1-2 Les empreintes et les traces dans l'enquete criminelle. 3-4 Les preuves de l'identité. 5-6 L'expertise des documents*

écrits. Les correspondances secrètes. Les falsifications. [Forensics: 1-2 Footprints and traces in the criminal investigation, 3-4 Evidence of identity. 5-6 The expertise of written documents. Secret correspondence. Falsifications.], vol 1-6. J. Desvigne et ses fils, Lyon.

Miller JD (2018) The role of dust mites in allergy. *Clinical Reviews in Allergy and Immunology* 25: 1-18.

OConnor BM (2009) Astigmatid mites (Acari: Sarcoptiformes) of forensic interest. *Experimental and Applied Acarology* 49: 125-133.

OConnor BM, Pimsler ML, Owings CG and Tomberlin JK (2015) Redescription of *Myianoetus muscarum* (Acari: Histiostomatidae) associated with human remains in Texas, USA, with designation of a neotype from Western Europe. *Journal of Medical Entomology* 52: 539-550.

Ostojic L and Wurmbach E (2017) Analysis of fingerprint samples, testing various conditions, for forensic DNA identification. *Science and Justice* 57: 35-40.

Perotti MA (2009) Mégnin re-analysed: the case of the newborn baby girl, Paris, 1878. *Experimental and Applied Acarology* 49: 37-44.

Perotti MA, Goff ML, Baker AS, Turner BD and Braig HR (2009) Forensic acarology, an introduction. *Experimental and Applied Acarology* 49: 3-13.

Perotti MA and Braig HR (2019) Acarology in crimino-legal investigations: the human acarofauna during life and death. In: Byrd JH and Tomberlin JK (eds) *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. 3rd edn. Taylor & Francis, Boca Raton.

Petraco N and Kubic T (2004) *Color Atlas and Manual of Microscopy for Criminalists, Chemists, and Conservators*. CRC Press, Boca Raton.

Poy AL and van Oorschot RA (2006) Trace DNA presence, origin, and transfer within a forensic biology laboratory and its potential effect on casework. *Journal of Forensic Identification* 56: 558-568.

Prussin AJ and Marr LC (2015) Sources of airborne microorganisms in the built environment. *Microbiome* 3: 78-88.

Ramirez CR and Parish-Fisher C (2017) *Crime Scene Processing and Investigation Workbook*. CRC Press, Boca Raton.

Rivers DB and Dahlem GA (2014) *The science of Forensic Entomology*. Wiley Blackwell, Chichester; pp 382.

Siegel JA and Mirakovits K (2015) *Forensic Science: The Basics*. 3rd edn. CRC Press, Boca Raton.

Smith KGV (1986) *A Manual of Forensic Entomology*. British Museum (Natural History), London; pp 205.

Solarz K (2009) Indoor mites and forensic acarology. *Experimental and Applied Acarology* 49: 135-142.

Stoney DA and Stoney PL (2015) Critical review of forensic trace evidence analysis and the need for a new approach. *Forensic Science International* 251: 159-170.

Szalanski AL, Austin JW, McKern JA, McCoy T, Steelman CD and Miller DM (2006a) Time course analysis of bed bug, *Cimex lectularius* L., (Hemiptera: Cimicidae) blood meals with the use of polymerase chain reaction. *Journal of Agricultural and Urban Entomology* 23: **237-241**.

Szalanski AL, Austin JW, McKern JA, Steelman CD, Miller DM and Gold RE (2006b) Isolation and characterization of human DNA from bed bug, *Cimex lectularius* L., (Hemiptera: Cimicidae) blood meals. *Journal of Agriculture and Urban Entomology* 23: 189-194.

Szelecz I, Lösch S, Seppey CVW, Lara E, Singer D, Sorge F, Tschui J, Perotti MA and Mitchell EAD (2018) Comparative analysis of bones, mites, soil chemistry, nematodes and soil micro-eukaryotes from a suspected homicide to estimate the post-mortem interval. *Scientific Reports* 8: 25.

Szkuta B, Harvey ML, Ballantyne KN and van Oorschot RA (2015) DNA transfer by examination tools–a risk for forensic casework? *Genetics* 16: 246-254.

Thomas WR (2010) Geography of house dust mite allergens. *Asian Pacific Journal of Allergy and Immunology* 28: 211-212.

Toothman MH, Kester KM, Champagne J, Cruz TD, Street IV WS and Brown BL (2008) Characterization of human DNA in environmental samples. *Forensic Science International* 178: 7-15.

Turner BD (2009) Forensic entomology: a template for forensic acarology? *Experimental and Applied Acarology* 49: 15-20.

Valerio CR, Murray P, Arlian LG and Slater JE (2005) Bacterial 16S ribosomal DNA in house dust mite cultures. *Journal of Allergy and Clinical Immunology* 116: 1296-1300.

White PC (ed) (2016) *Crime Scene to Court: The Essentials of Forensic Science*. 4th edn. Royal Society of Chemistry, Cambridge.

Wickenheiser RA (2002) Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. *Journal of Forensic Sciences* 47: 442-450.

Wyss C and Cherix D (2013) *Traité d'entomologie forensique. Les insectes sur la scène de crime [Treatise on forensic entomology. The insects at the crime scene]*. Collection Sciences Forensiques. Presse polytechniques et universitaires romandes, Lausanne.

Zubakov D, Chamier-Ciemińska J, Kokmeijer I, Maciejewska A, Martínez P, Pawłowski R and Kayser M (2018) Introducing novel type of human DNA markers for forensic tissue identification: DNA copy number variation allows the detection of blood and semen. *Forensic Science International: Genetics* 36: 112-118.

4 An analysis of microsatellites in mites

4.1 Introduction

Microsatellites are simple sequence tandem repeats (SSRT) of nucleotides that are polymorphic in nature and can be used to determine a genetic linkage between two or more individuals. The repeated nucleotide sequences may vary in number across different individuals making the method suitable for conducting genetic fingerprinting (Goldstein and Schlötterer, 1999; Butler, 2005; Kantartzi, 2013; Goodwin, 2016; Schausberger et al., 2016; Bagshaw, 2017). Since its discovery in the 1980s, genotyping with microsatellites has become a widely accepted tool for conducting research. In medicine, microsatellites are used for predicting therapeutical effectiveness of certain groups of medication, for example for malignant diseases like Lynch Syndrome (Andre, 2019). Forensic investigations have benefitted from genotyping with microsatellites because it not only provides accurate results but can also be found in abundance. In cases where parenting is in doubt, microsatellites can be used to determine the biological parent of a child. In agriculture, genotyping of microsatellites has been used to create pest resistant plants that do not require pesticides or herbicides. Genotyping with microsatellites has also been used in analyzing organisms such as mites to understand the genotypic differences between various populations.

4.2 Microsatellites in mites

The genetic structure of mites has attracted significant number of studies. Most of the studies have focused on how separation of mites into different geographical regions or host species affect their genetic structure. Since some species of mites are potentially destructive to plants and animals, researchers have focused on how the genetic structure can be used in managing destructive mites. Genetic variations in mites has been a significant concern among researchers because it determines the ability of mites to resist acaricides. For instance, a pesticide used in killing mites in Europe, may not have similar impact in Asian countries due to geographical distance. Genotyping with microsatellites has been useful towards determining the heterozygosity among similar species of mites obtained from different regions. Examples of the application of microsatellites are given in Table 4.1.

Microsatellite in mites can be simple sequence tandem repeats. The repeats can occur in the form of di, tri, or multiple sequences. For example, if AC is the repeat chromosome determines the genetic characteristics of the organism. Microsatellites are motif, the number of times letter A or C repeats in a particular valuable genetic marker because they are highly polymorphic. In other words, a single mite can have 10 or 15 alleles and heterozygosity above 0.85. The reason behind high polymorphism is the systematic mutation patterns. Microsatellites do not mutate using classical patterns (Guichoux et al., 2011). A classical mutation pattern is where letter 'A' in an 'AB' dinucleotide is replaced with 'G' to form a 'GB' dinucleotide. In this type of mutation, the genetic characteristics may change completely. The microsatellite mutation process is known as the slippage replication. For example, when analyzing a repeat unit such as 'AT', it is possible that two strands can slip positions but still manage to keep the chain going. This can result into a chain with longer 'T' and shorter 'A' or another chain with shorter 'T' and longer 'A'. This type of mutation may transform the genetic characteristics of organisms within the same species. Mites become a good example when analyzing the impact of the mutation process in microsatellites.

Habitat fragmentation or the transfer of mites from one geographical region to another can affect population structure. Microsatellite markers can reeal the host taxon-derived characteristics among *Sarcoptes* mites (Acariformes: Sarcoptidae) obtained from three European countries, 15 wild mammal populations belonging to 10 host species. A total of 10 markers relevant to *Sarcoptes* mites were used to apply microsatellite genotyping. The results showed a significant genetic variation among the *Sarcoptes* mites obtained from different geographical regions. Wild host-derived populations were grouped into herbivores, omnivores and carnivores depending on their eating habits. The omnivore group was located halfway between the carnivores and herbivores. The differences between the three mite groups was supported by their geographical separations. In other words, limited or lack of gene flow between *Sarcoptes* mite populations influenced parasitic adaptations as well as the creation of host taxon-derived characteristics.

Application of microsatellites in Acari

Study object	Species	Region	# MS	Notes	Reference
Agriculture					
Spread of acaricide resistance	*Tetranychus urticae*	China	5	Resist. evolved locally	(Shi et al., 2019)
Reservoir plants	*T. turkestani*	France	5	Weed is reservoir homozygocity	(Bailly et al., 2004)
Invasion of honey bee brood cells	*Varroa destructor*	USA	6	Random	(Beaurepaire et al., 2019)
Introduction		Madagascar	11	Low diversity	(Rasolofoarivao et al., 2017)
Veterinary Medicine					
Population structure	*Dermacentor reticulatus*	Europe	5	Two pop.: West, East	(Paulauskas et al., 2018)
Population structure	*Rhipicephalus microplus, R. decoloratus*	South Africa		Homogenisation pop. cattle movement	(Baron et al., 2018)
Human Medicine					
Population structure	*Sarcoptes scabiei* var. *hominis*	Pakistan		Genetic isolation host sp, geography	(Naz et al., 2018)
Population size estimates					
	Amblyoma variegatum	Burkina Faso	8	Very small	(Huber et al., 2019)

Table 4.1 – Examples of the application of microsatellites in the study of Acari.
 # MS: Number of microsatellites used in study.

If same population of mites are separated into different geographical regions, they will develop unique features to sustain their survival in such regions. The unique features create a discontinuity in the genetic patterns that can be observed between the mite populations. For example, in the same population, some mites are likely to become herbivores, carnivores or omnivores based on their host environments. One of the ramifications of this findings may be reflected in the genetic structure of the populations, life cycles, diagnosis and the monitoring protocols. The *Sarcoptes* mites, for instance, are known for causing skin itches or scabies in the wild animal populations or human beings. Researchers have found some elements of resistance when similar pesticides are used across different geographical regions. This kind of study associates such resistance to the unique genetic characteristics that mites are capable of developing when separated in different geographical regions.

The impact of habitat fragmentation on microsatellite variation has been studied in the spider mite pest *Tetranychus turkestani* (Acariformes: Tetranychidae). The researchers sampled a total of 283 spider mites collected from 15 different hosts consisting crops and weed in southern France (Bailly et al., 2004). A total five microsatellite markers were used to determine the genetic characteristics within the population. The researchers found significant genetic variations with an average heterozygosity of 0.68. While the variations in genetics could be attributed to different geographical environments, the researchers could not find significant differences between mites living on different host plants. Mites collected from the same host plant but in different geographical locations showed 97 % differences in the genetic characteristics. Moreover, in 11 out of the 15 samples collected, the researchers found significant heterozygocity. The findings in this research support previous conclusions that plants in a given locality are colonized by mites. Another conclusion support by the researchers is that inbreeding may enhance homozygosity among mites feeding on different plants. In agriculture, the findings inform farmers that mites have the capacity of continuous survival by moving from plants to weeds. When applying pesticides, farmers should not only focus on the plants but also on the weeds.

Characteristic of microsatellites so far developed for Acari are listed in Table 4.2.

Development of microsatellites in Acari

Genomic approaches								
Species	**Method**	**State**	**Repeats**	**Repeats**	**Flank**	**Seize**	**Tail**	**References**
Tetranychus urticae	WGS	P	di, tri, tetra, penta	≥ 11	(200)	80-300	M13	(Ge et al., 2013)
							fluor. C	(Shi et al., 2019)
Enriched libraries								
Species	**Method**	**State**	**Motifs**	**Repeats**	**Flank**	**Seize**	**Tail**	**References**
Tetranychus urticae	FIASCO		CT, CTT, GT, CAC	4-33		52-249	M13	(Sabater-Muñoz et al., 2012)
			CA, CT	2-17		95-350	varies	(Uesugi and Osakabe, 2007)
Aceria tosichella				≥8		155-298		(Miller et al., 2012)
Colomerus vitis			AC, AG	9-24		87-157		(Carew et al., 2004)

Table 4.2 – Examples of the detailed characteristics and methods used to develop microsatellites in Acari. Only once were microsatellites developed by whole genome sequencing, all other microsatellites were developed with a predetermined motif.
State P: Perfect; **IP:** Imperfect; **Flank:** Flanking region either side of repeats in bp (primer design region); **Seize:** Average seize of amplicon in bp. **PP:** Primer pairs; **PCR:** PCR-positive primer pairs; **PMS:** Polymorphic microsatellites; **Allel.:** number of alleles per microsatellite.

Microsatellite genotyping has also been useful in the study of genetic epidemiology and pathology of the certain disease-causing mites such as *Sarcoptes* mites. The study conducted by Renteria-Solis and his colleagues focused on investigating *Sarcoptes* mite infections in raccoons in the German cities of Berlin and Kassel. The research also focused on determining the putative origin of the parasite. Although Raccoons are common in the rural areas, they are considered invasive in most urban centers across Europe. In Germany, raccoons mostly live in the rural areas and dense forests. However, recent changes in their primary habitats have seen some raccoons migrating to the urban centers. The cities of Kassel and Berlin have played host to a significant number of raccoons. In this study, the researchers were concerned about the presence of mite infections in most raccoons living in the cities. Also known as scabies, *Sarcoptes* mite infections can causes serious complications and even death in some cases. To determine the putative source of the infections and parasite morphology, the researchers relied on nine microsatellite markers to genotype individual mites from five raccoons and compare results with *Sarcoptes* mites obtained from foxes, wild boar and Northern chamois obtained from Italy and Switzerland respectively. The raccoon-derived mites clustered together with the samples obtained from fox but differed significantly from the samples obtained from wild boar and chamois.

In this case, the results point towards fox as the putative source of the *Sarcoptes* mite infections. The results also indicate the impact of geographical fragmentation or isolation in the genetic characteristics of the mites. For instance, the researchers are capable of clustering *Sarcoptes* mite samples obtained from foxes and raccoons living in the same geographical region. However, the samples differ significantly from the *Sarcoptes* mites obtained from Italy and Switzerland. The results are consistent with the previous findings obtained from different researchers. Such consistency also provides insights into the accuracy of microsatellite genotyping. The researchers concluded that foxes were the origin of the mite infections. However, they could not tell whether the interaction between foxes and raccoons were the cause of the infections. The conclusion was driven by the genetic characteristics between the *Sarcoptes* mites obtained from the raccoons and foxes.

However, the genetic characteristics in the *Sarcoptes* mites obtained from wild boar and chamois could not match the ones in Germany due to geographical differences. The results can be used in the management of scabies infections by treating foxes which seem to be the origin in Germany. Therefore, the genetic epidemiology and pathology of the raccoon-derived *Sarcoptes* mites can be understood and managed based on their geographical locations.

The adaptability of mites to their host environments is one of the main reasons behind genetic diversity. The genetic variations in mites is a problem that has affected many farmers across the world. A good example is the case of ectoparasitic mite *Varroa destructor* (Parasitiformes: Varroidae), which is a major global threat to the Western honeybee *Apis mellifera* (Hymenoptera: Apidae). The parasitic mite *V. destructor* was an original parasite of *A. cerana* which is a native of Asia. However, to improve the production of honey in the continent, farmers introduced *A. mellifera*. Within a reasonable period, V. destructor was able to spill over to the colonies of *A. mellifera* because of proximity. The spill over and spill backs that have been observed in *V. destructor* has caused panic among farmers because of the unpredictable consequences on the honeybees. There have been cases where production is limited by the parasitic attacks. What makes the control even more difficult is the ability of the Varroa destructor to adopt the conditions of its host before colonizing it. Therefore, to determine the impact of host specificity in *V. destructor*, two samples of mites were obtained from Vietnam and Philippines. The researches focused on the dispersal and hybridization potential of *V. destructor* from the colonies of the two hosts found in Northern Vietnam and the Philippines.

Using mitochondrial and microsatellite DNA markers, the researchers found significant disparity in the genetic characteristics of the *V. destructor* obtained from the two hosts. The researchers found different mtDNA haplotype equally invading *Apis cerana* and *A. mellifera* in Philippines. In Vietnam, there were completely different *Varroa* populations in *A. cerana* and *A. mellifera* even if they were kept in the same colonies. The difference in the two sets of *Varroa* populations indicate the role of host on determining the genetic diversity among mites. The results indicate that mites have the capacity to adopt conditions which they believe can enhance their

survival in a particular host. Once the adaptable characteristics have been developed, they are incorporated into the DNA and become genetic characteristics. The features can be transferred from one generation to another to sustain future populations. This becomes a challenge to farmers because the adaptable features can also increase resistance towards commonly used pesticides. Once the mites have adapted to their host, they become part of it and may use the acquired traits to overcome threats such as pesticides. This explains why researchers must study the origins of parasitic mites to determine suitable control mechanisms.

Microsatellite genotyping has also been used to determine factors that drive polyandry in predatory mites. While most female mites tend to be monandrous, male mites are generally polygynous. Being monandrous denies female mites the ability to sire offspring that can survive in difficult environments. A study was conducted to determine factors that drive polyandry among predatory mites. In the experiment, there were two sets of mites in which one was highly polygynous (up to 45 fertilized females in life) and a less polyandrous mite (up to eight male mates in life). The highly polygynous mite was known as *Neoseiulus californicus* (Parasitiformes: Phytoseiidae), while the less polyandrous female was known as *Phytoseiulus persimilis* (Parasitiformes: Phytoseiidae). The researchers applied microsatellite genotyping to determine the genetic characteristics of the two sets of offspring. The results indicated that multiply mated females produced offspring with higher chances of survival while singly mated females produced offspring with limited chances of survival. In the case of multiple mating, the females produced offspring with paternities that enhance chances of survival. The indirect benefits associated with the polyandry include enhanced genetic compatibility and variability. The genetic compatibility is what assist offspring to survive difficult environmental conditions. The microsatellite genotyping assists researchers to identify specific genetic characteristics that act as the ultimate drivers towards polyandry among predatory mites.

Not all mites have the same number or pattern of the microsatellite sequence. Irregular patterns of microsatellite sequences were found in two mite genomes. The two species that were tested include *Tetranychus urticae* and *Amblyseius fallacis*. The

research was conducted by screening of both mite genomic libraries and southern blots of the mites before comparing them to two vertebrates. There were no signs of GT15 or CT10 in the two mite species. However, the two probes were strongly found in the vertebrates' DNA. The missing sequence cane be used to tell the difference between vertebrates and mites. The scarce repetitive DNA isolated from the two mite species can be used as population markers. Researchers can rely on the two probes to tell the genetic difference between mites and other species living in the same habitat. The results also indicate a significant case where microsatellite genotyping can assist researchers in identifying population markers that can be used for isolations.

Mites are unique individuals based on their genetic diversity. A lot of research has been conducted to determine how this significant variation in the genetic characteristics can be used in the management of parasites that are destructive to plants and animals. For instance, parasitic mites such as *Sarcoptes* mites can be analyzed based on its genetic characteristics in different locations. Spider mites are known for the extensive adaptive characteristics that enable them to feed on various plants within their habitat. Even if removed from plants using pesticide or insecticides, spider mites can still survive in different hosts including weeds. The study of their genetic structure enables scientists to discover various strategies that can be used to preserve harmless mites and destroy mites that can be harmful economically or to human health.

While human features can be observed using physical and biological characteristics, mites are so tiny that only microsatellite genotyping can assist researchers to determine genetic variations. The results have been used to determine reasons behind pesticide resistance among spider mites or *Sarcoptes* mites. The differences in genetics can assist mites to survive in different environments by developing adaptive characteristics. Farmers have also benefitted from the microsatellite studies because they can improve their pest control strategies. Research indicates that mites are capable of colonizing weeds once they have been removed from the plants. Once the effects of the pesticides decline, they simply

return to plants and the problem continues. However, if farmers also focus on the weeds, they may improve the quality of pest control strategies.

In an analysis of only perfect microsatellite loci obtained through a whole genome survey in *Tetranychus truncatus*, only one out of 36 microsatellites should signs of positive selection, meaning a single allele of that locus is involved in a function beneficial for the individuals in a population, often leading to homozygotes in that locus to be fitter than heterozygotes, and only one other one showed signs of balancing selection, meaning that multiple alleles of this locus are more common in a population than expected from genetic drift alone and therefore considered positively selected as well, often leading to heterozygotes in that locus to be fitter than homozygotes; all other 34 microsatellites seem to under no selection and can evolve unrestricted (Ge et al., 2013), Figure 4.1.

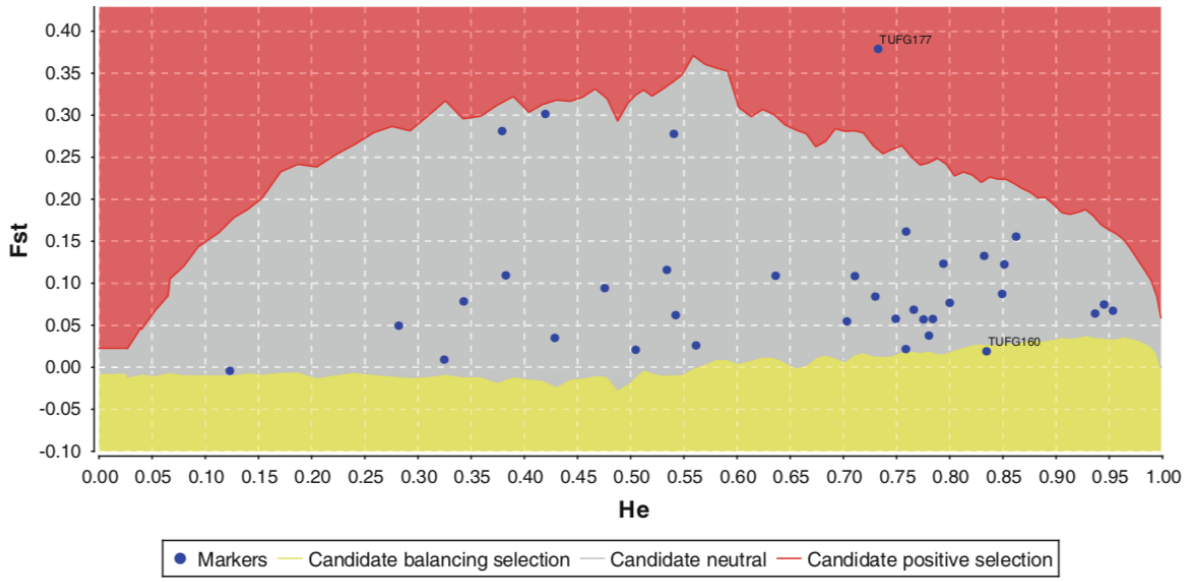


Figure 4.2 – Thirty four out of 36 microsatellite loci in *T. truncates* show no signs of selection (grey), one positive selection (red), and one balancing selection (yellow) (Ge et al., 2013). Analysis using F-statistics implemented in LOSITAN. Fst is the proportion of the total genetic variance contained in a subpopulation S, relative to the total genetic variance T. The higher the Fst value, the more structured the subpopulations is. He: expected heterozygosity.

4.3 Success rates of microsatellite design

Microsatellite designs have been used for nearly three decades since their discovery in the 1980s. Although several technologies have been developed with the aim of improving or replacing the microsatellites, they are still useful in many experiments. The ideal features of a genetic marker that scientists may be looking for include polymorphism, codominance, and even distribution throughout the genome. Other features include low cost, high-throughput, reproducibility, and the ease of transfer between laboratories or populations. Most genetic markers used in the past and present do not meet all the characteristics above. In most cases, a single genetic marker can have two or more characteristics described above.

One of the key factors behind success rates of microsatellite design is reproducibility. Reproducibility is the ability to obtain the same results from the same DNA template. In experiments where accuracy is required, a reproducible genetic marker is not only convenient but also significant in producing convincing outcomes. For example, the same DNA template can be taken to different

laboratories for confirmation. While reproducibility of the microsatellite profiles is similar to that of other genetic markers including RFLPs, SSR analysis are simple and only require a small amount of DNA template. The simplicity in the extraction of the DNA samples enable researchers to obtain templates even from highly decomposing tissues. The SSR analysis does not require ultra-pure DNA templates. The experiment can work even in the presence of a few impurities. The SSR analysis does not require the use of enzymes to restrict or achieve specific outcomes. The use of enzymes may create impurities that can interfere with the quality of outcomes. Without enzymes, the same DNA template can be used in different types of experiments without compromising the quality of the results.

In many genetic markers including the SNPs, the use of contaminated DNA templates must be supported by enzymes to minimize discrepancies. Obtaining an ultra-pure DNA template can be a challenge especially when dealing with highly decomposing tissues. In the case of mites, contamination can be caused by overflow of undesired traits or environmental features such as pollution. If enzymes are to be used in conducting the experiments, the cost may rise significantly and the templates cannot be reused to confirm the outcomes. The SSR analysis has been used in the analysis of fossils due to their reproducible capacity. Even the mummified fossils obtained from places such as Egypt have been analysed using the SSR designs. Challenges that may arise while using the SSR designs can be solved by focusing on the mapped markers that are easy to verify. The mapped markers provide accurate details about genetic characteristics enabling researchers to overcome challenges that can be caused by the contamination of the DNA templates.

Another factor that influences the success rate of microsatellite designs is being highly polymorphic. The SSRs are highly variable evidenced by the high allelic variations even among the closely related species. For example, researchers have identified significant allelic variations between *Sarcoptes* mites found in Germany and the same species obtained from wild animals in Italy and Switzerland. Allele is a viable DNA coding that determines the features of a chromosome. In other words, it carries information that are used to create the proteins making up particular genes (Sme et al. 2018). Alleles can be the same or different depending on

the nature of the sources. For example, an allele can carry similar information concerning the eye colour from the mother and father of an offspring. Homozygous alleles contain the same information from the parents. For example, if both father and mother have brown eyes, the allele will carry the same information to the chromosomes. If the father has blue eyes while the mother has brown eyes, the allele will carry different information from the two parents. This case is known as heterozygous alleles.

The SSR analysis offer an advantage because it provides high allelic variations that can be observed to determine the linkage mapping. The number of alleles in a typical SSR profile may vary from 1 to 37 with a diversity or heterozygosity indices varying from 0.25 to 0.95. When compared to other genetic markers, the level of variation is almost twice or three times indicating a great advantage in favour of SSR analysis. Unlike human beings, the study of mites requires genetic markers that can tell the variations between species bearing similar genes. However, in SSR analysis, bands produced from the same primers are usually orthologous. In genetics, orthology can be achieved during the specialization of genes. A good example is the case of host-derived characteristics in mites.

The co-dominant nature of the SSRP profiles makes them suitable for studying segregation in the F2 populations. While studying mites, F2 population can be obtained by crossing mites and putting their offspring in two different hosts. After a specified period of time, the two species can be subjected to scientific tests to determine genetic variations. Parentage analysis in hybrids can also be determined using the co-dominant features. The co-dominant features can be used to determine the impact of geographical or climate changes to the mite populations. Researchers can compare and contrast populations that existed in the 20th Century to the current population to determine the impact of climate change on the survival of mites. The adaptive features identified in such experiments can be used in breeding to enhance the survival of the future generations.

Studies conducted on various eukaryotic species indicate that SSRs are sufficiently abundant across the genomes. Nearly every part of the body can be used to conduct SSR analysis. In the recent studies, researchers found that SSRs are

abundantly available in the faeces. This has made experiments much easier and less expensive. Researchers can easily obtain DNA samples even from the faeces of animals or the remains of plants. This may avoid the use of live animals in conducting experiments. Over the years, concerns have been raised concerning the use of live animals or extraction of DNA samples from the bone marrow tissues. When cornered, most researchers have not been able to explain what they do with animals usually discarded after experiments. The use of live animals can be avoided by obtaining DNA samples from wastes such as faeces. The cost of experiments may also reduce if researchers rely on materials that are not only cost effective but also provides the desired outcomes. The abundant nature of the SSRs even in highly decomposing tissues is a great advantage that microsatellites have over other genetic markers.

Researchers argue that the SSR markers are preferentially associated with the non-repetitive DNAs. With conducting the SSR analysis, the genomic sites of the SSR markers mainly fall into the transcribed regions in the case of genic SSR markers. However, in the case of the genomic SSRs, the markers are likely to fall in the non-transcribed regions. The cDNA or the EST contain genic SSRs that can be used in conducting gene tagging or gene function characterization. A gene function characterization enables researchers to map genes with their specific roles in an organism. In agriculture, the gene tagging or gene function characterization can be used to identify suitable genes that can enhance the survival of plants in specific conditions. For example, genes that support plants such as cactus and enhance their survival in arid areas can be used in developing drought resistant organisms. In the case of mites, gene function characterization can be used to identify adaptive features that enable mites to survive in different colonies. However, the research on non-repetitive DNAs has not been extensive because some of the EST or cDNA sequences are not freely accessible to the public.

4.4 Characteristics of successful microsatellites

Amplicon length: The amplicon length is an important parameter in the polymerase chain reactions (PCR). Other important parameters include Tm of the primers and

probe. The amplicon is a piece of DNA or RNA obtained after amplification or replication. The amplification can be done through polymerase chain reactions or the ligase chain reactions. In many experiments, the optimal amplicon length should be less than 150 bp. However, to obtain more precise outcomes, the recommended amplicon length should be less than 80 bp, with 50 bp being the most appropriate length. The shorter amplicon length is more appropriate because it amplifies more efficiently and highly tolerant to suboptimal conditions. The amplicons are usually denatured between 92 and 95 °C of the PCR allowing primers and probes to enhance accuracy by complementing the experiments.

Examples of the numbers of polymorphic microsatellites finally developed for certain mite species are noted in Table 4.3.

The primary role of the amplicon length is to enable researchers study the allelic variations in a piece of a DNA template. Since microsatellites are PCR enabled, they allow researchers to accurately observe the genetic characteristics in a piece of the DNA. Keeping the length below 80 bp enables researchers to avoid constraints or dimers that occur when the primers are stretched beyond their elastic capacities. Besides, the amplicon length enables researchers to use only a small piece of the DNA template to study the genetic characteristics. Amplicon length also provides a great advantage when dealing with decomposing tissues. Only a small piece of the tissue is required to accurately observe the genetic characteristics.

Success of microsatellite development in Acari

Species	Clo	PCRClo	PP	PCR	(P)MS	Allel.	AvAll.	TAll	PIC	Range	References
Tetranychus truncatus			205	102	36	2-23	8.7	313	0.589	0.119–0.922	(Ge et al., 2013)
T. urticae	1,786	407	34	22	11	1-5					(Sabater-Muñoz et al., 2012)
		46	34		16	2-5					(Uesugi and Osakabe, 2007)
Aceria tosichella		192	40	15	9	8-15					(Miller et al., 2012)
Colomerus vitis			10		4	1-14					(Carew et al., 2004)

Table 4.3 – Examples of the success rate of developing microsatellites. In some examples, the authors call a microsatellite with one allele still a microsatellite, which is obviously not polymorphic and of no use.
Clo: Number of clones in enriched libraries; **PCRClo:** PCR selected clones; **PP:** Primer pairs; **PCR:** PCR-positive primer pairs; **(P)MS:** Polymorphic microsatellites; **Allel.:** number of alleles per microsatellite; **AvAll:** Average number of alleles per microsatellite; **Tall.:** Total number of microsatellites; PIC: Polymorphic information content of microsatellite; Range: Range of PIC.
Tk Mean observed heterozygocity 3 pop. 0.468, expected 0.628.

The repeat length: The repeat length of the microsatellites is determined by the mutational mechanisms. Gene mutations can lead to an increased frequency of repeats or decreased frequency. An increased frequency would lead to a longer microsatellite while a decreased frequency may shorten the length of the microsatellites. Researchers are interested in the repeat length because it provides rich allelic variations. So many alleles can be observed in a longer microsatellite than a shorter profile. In cases where the repeat length is longer, researchers are likely to obtain accurate results than in the reverse situations. Researchers are also interested in the repeat length because it determines the genetic distance measure that can be used to study the characteristics of a population. The genetic distance measure can be used to study relationships between two species of mites to determine environmental or host-derived characteristics. The abundance of alleles also enables researchers to identify signs of a genetic disorder. For instance, a longer repeat length may signal the occurrence of a malignant tissue or discontinuity of a vital genetic feature. Therefore, the repeat length contributes towards increasing the accuracy of SSR analysis.

Repeat type: The repeat types come in the form of di-, tri- and tetra-nucleotide repeats. A series of tandemly repeated units is what defines a microsatellite. Each microsatellite may have at least five or more tandemly repeated units. Moreover, mutations that occur along the polymerase chain may determine the repeat type as well as the nature of the microsatellites.

One of the roles of the repeat type is to establish a pattern or sequence that can be studied in two or more species. Microsatellites provide more advantage because the sequence is conspicuous and can be easily established (Salinas-Vargas et al. 2016). The polymerase chain reaction (PCR) increase the amplicon length and enables researches to observe microsatellite sequence. At least five or more tandemly repeated units can be observed in a single microsatellite. The repeated units are used in creating linkage maps needed in making logical conclusion. The linkage map shows the relationship between DNAs obtained from different species. The relationship is positive if the DNA sequence of one species accurately maps on to the DNA sequence of another species. The relationship may return negative in

the case of discrepancies in the DNA sequence. The type of repeats may also tell researchers about the potential occurrence of a malignant tissue. The tri-nucleotide repeat, for instance, is associated with the human disorders such as fragile X syndrome as well as the myotonic dystrophy. Not much has been found about the effects of triplet repeats on the genetic characteristics of mites.

Perfectness: For genetic markers to be approved by researchers or government institutions, it must provide perfect outcomes. The DNA experiments are used in fingerprinting to determine the identity or relationships between two or more individuals. Fingerprinting is one of the methods used by police officers to determine the identity of their suspects or convicts. If the results are inaccurate, the police will get a wrong identity and this can lead to a wrongful conviction. Several mistaken identities have occurred due to faults in the machines used for conducting fingerprinting. Apart from fingerprinting, the DNA experiments have been used in the diagnosis of diseases. Most cancerous tissues in the body are determined using DNA analysis. The problem can occur when a doctor gives wrong information concerning the condition of a patient. For example, if the doctor wrongfully diagnoses a patient with cancer, such a mistake may have far reaching mental and physical consequences.

The best genetic marker is selected based on the accuracy of the outcomes. Factors such as human error may affect the accuracy of the outcomes. Microsatellites have minimal human interference and may have little or insignificant error. Human error may occur because microsatellite analysis is usually semi-automated. Humans do the work of extracting DNA tissues, preparing them for the experiments by cutting them into sizes through a process known as fragmentation. The fragmented pieces are then placed on nylon membranes in a process known as plating. Therefore, between DNA extraction and plating, researchers may make mistakes that can affect the final outcomes. In comparison, the SNPs have limited errors because the processes are fully automated. The only advantage that microsatellites have over SNPs is the possibility of using even slightly contaminated DNA templates and still getting accurate results. Table 4.4 gives a representative overview of microsatellites in mites.

Repeat motifs of microsatellites in Acari

Unbiased, perfect

	AG/CT	AC/GT	GA/TC	References
Tetranychus T. truncatus	14, 13, 12, 12, 12 16, 11, 11, 12, 12 14, 18, 11, 23, 11 27, 13, 11	11	11, 13, 11, 11, 25 29, 28, 13, 18, 13 11, 13, 11	(Ge et al., 2013)

	ATA/TAT	ATC/GAT	AGA/TCT	GAA/TTC	References
T. truncatus	11	13	11	11	(Ge et al., 2013)

Biased

	AG/CT	AC/GT	GA/TC	CA/TG	References
T. urticae		21, 33			(Sabater-Muñoz et al., 2012)
	8, 15, 9, 9	6, 6	13, 7, 17, 8, 9	7, 7, 7	(Uesugi and Osakabe, 2007)
Colomerus vitis	12, 24	10	9	10	(Carew et al., 2004)

	ATA/TAT	ATC/GAT	AGA/TCT	GAA/TTC	CAC/GTG	CTT/AAG	ACC/GGT	GAA/TTC	References
T. urticae			6		8, 8, 9, 9	9, 8	4, 4	4	(Sabater-Muñoz et al., 2012)
T. urticae	Imperfect (CA)2A(CA)7		(TC)7C(TC)5						(Uesugi and Osakabe, 2007)

imperfect		
Mite species		
Vernacular name or life style	**Family, Order**	**Superorder**
Microsatellite reference		
microsatellites discovered, repeats, length, where available		
1. *Neoseiulus californicus* (McGregor, 1954)		
Predator mite	Phytoseiidae, Mesostigmata	Parasitiformes
Seiter and Schausberger (2015) 10 microsatellites, 3 polymorphic $(GA)_8$, $(AG)_3GG(AG)_7$ 172, 237 bp Perrot-Minnot, Lagnel, Desmarias, and Navajas (2001) 5 site-specific microsatellites were used 5'TTTCGGTGTTCGCATTTCG3',5'ACACCGCAGTCAGTCAAGC3',5'TCCCTGAGTGGCTTCGAG3',5'CCGAAAGACAACTCGGCAT3',5'GGGTTCAAACTTCAGACCC3'		
2. *Neoseiulus womersleyi* (Schicha, 1975)		
Predatory mite		
Predatory mite Hinomoto et al. (2011) 10 microsatellite markers developed for population study $(CT)_{16}$, $(AG)_{11}$, $(CT)_{23}$, $(TC)_9$, $(GA)_8$, $(CT)_{12}$, $(TTTCTCTC)_{26}$, $(CT)_{10}$, $(GA)_{24}$, $(CT)_{23}$ Hinomoto and Maeda, (2005) 3 microsatellites were isolated. $(GA)_{19}G$, $CC(GC)_3(TC)_{11}$, $(GT)_2(TG)_7(CG)_4(AG)_3(TG)_2$		
3. *Phytoseiulus persimilis* (Athias-Henriot, 1957)		
Predator mite	Phytoseiidae, Mesostigmata	Parasitiformes
Schausberger et al. (2015)		

10 microsatellites, 6 polymorphic $(CT)_{10}$, $(GA)_{10}$		
4. *Spinturnix myoti* (Kolenati, 1856) and *S. bechsteini* (Deunff, Walter, Bellido & Volleth, 2004)		
Bat mite	Spinturnicidae, Mesostigmata	Parasitiformes
Schaik et al. (2011) 5 highly polymorphic markers $(GT)_{17}$, $(CA)_{19}CG(CA)_{11}$, $(GT)_{31}$, $(CA)_{17}(CAA)_5$, $(CA)_{11}AA(CA)_{13}$		
5. *Varroa destructor* (Anderson & Trueman, 2000)		
Varroa mite	Varroidea, Mesostigmata	Parasitiformes
Solignac et al. (2005) 16 microsatellite markers $(GA)_{10}$ $(GA)_6(GA)_5$, $(GT)_{17}$,$(TC)_7GC(TC)_5$, $(AC)_8$, $(TC)_{16}(AC)_8(TC)_4$, $(CT)_{14}$, $(GT)_{10}$, $(AT)_6(TG)_9$, $(CA)_7$, $(AG)_{13}$, $A_9(CA)_8$, $(AC)_{10}$, $(AC)_7GT(TA)_4$, $(CA)_{10}(CA)_4$, $(CA)_{11}$, $(CA)_{10}$ Beaurepaire & Krieger (2017) 4 microsatellites $(TTA)_{12}$...$(AT)_7$, $(TAA)_6$...$(TA)_9$, $(AT)_8$...$(TA)_7$, $(TA)_{11}$		
6. *Varroa jacobsoni* (Oudemans, 1904)		
Varroa mite		
Evans (2000) 9 microsatellites developed. One locus failed to amplify in samples from certain regions (Russia and South Africa). $(GA)_9$, $(TC)_5Y_{14}(TC)_8$, $(TC)_{14}$, $(TC)_{23}$, $(AGC)_6AAC(AGC)_6$, $(CT)_{18}CC(CT)_5$, $(AG)_{23}$, $(TC)_{17}$, $(TC)_{19}$		
7. *Varroa* sp.		
Varroa mite		
Awad et al. (2010) 11 microsatellite primers were developed		

5'-CAGGCCCTTC-3', 5'-TGCCGAGCTG-3', 5'-AGTCAGCCAC-3', 5'-TGATCCCTGG-3', 5'-CATCCCCCTG-3', 5'-TGCGCCCTTC-3', 5'-TTCGAGCCAG-3', 5'-GTGAGGCGTC-3', 5'-GGGGGTCTTT-3', 5'-CCGCATCTAC-3', 5'-GATGACCGCC-3'

Kraus and Hunt (1995)

16 markers were used. Highly polymorphic and gave clear banding patterns

A3(5'AGTCAGCCAC),A6(5'GGTCCCTGAC),B14(5'TCCGCTCTGG),C12(5'TGTCATCCCC),H1(5'GGTCGGAGAA),J13(5'CCACACTACC),J16(5'CTGCTTAGGG),K11(5'AATGCCCCAG),P3(5'CTGATACGCC),W3(5'GTCCGGAGTG),X4(5'CCGCTACCGA).

8. *Psoroptes ovis* (Hering, 1838)

Sheep scab mite	Psoroptidae, Asigmata	Sarcoptiformes

Evans et al. (2003)

9 microsatellite markers

$(CA)_{13}$, $(CA)_{12}$, $(CA)_{9}$, $(TG)_{11}$, $(GT)_{11}$, $(GCT)_2(GTT)_1(GCT)_4$, $(GA)_{18}$, $(TTG)_5(CTG)_1(TTG)_3$, $(GA)_{13}$

Evans, Dawson, Wall, Burke, and Stevens (2004)

2 microsatellite markers

$(GA)_n$, [219 bp], $(ATT)_{n\ [243\ bp]}$

9. *Sarcoptes scabiei* (De Geer, 1778)

Itch mite	Sarcoptidae	Sarcoptiformes

Walton et al. (1997)

Panel of 10 microsatellites developd

(GA)n, (CT)n, (TC)n, (CTT)n, (GAAT)n, (TGT)nCNA(TGT)n, (GA)nCA(GA)n, (TAT)n, (CT)nTGGGGG(CT)n, (TTC)nT(TTC)n

Alasad et al, (2000)

1 microsataellite

(CT)n

10. *Aceria tosichella* Keifer, 1969

Wheat curl mite	Eriophyidae, Prostigmata	Trombidiformes

Carew et al. (2009)

2 primers developed

AAAGCCGTTTWACGCTAAGGTAGC, TACTCGACCCAAGTGATCCACCG

Jarman and Ward (2002)

2 microsatellite primers

TGCTTTCTCTACCCSTTSGACTTYG, CCAGACTGCATCATCATGCGWCGAC

Navajas et al. (1999)

1 microsatellite

AGAGGAAGTAAAAGTCGTAACAAG

Miller et al. (2012)

4 microsatellites

$(GA)_n$, $(CA)_n$, $(CT)_n$, $(TC)_n$

11. ***Eriophyes vitis*** **(Pagenstecher, 1857)**
Syn Colomerus vitis

Grapevine bud mite	Eriophyidae, Prostigmata	Trombidiformes

Carew et al. (2004)

5 microsatellites

$(CT)_{12}$, $(CT)_{24}$, $(GT)_{10}$, $(CA)_{10}$, $(GA)_9$

12. ***Halotydeus destructor*** **(Tucker, 1925)**

Red-legged earth mite	Penthaleidae	Trombidiformes

Hill et al. (2016)

$(CT)_{10}$,$(GA)_{10}$, $(AG)_{10}$,$(AG)_{11}$,$(AG)_{11}$,$(GA)_{11}$,$(GA)_{13}$,$(GA)_{14}$,$(CT)_9$,$(GA)_9$

40 microsatellites, 14 polymorphic (10 used), 26 monomorphic or failed to amplify

8-25 alleles per locus, mean 16, allelic richness 6.3-7.7

13. ***Polyphagotarsonemus latus*** **(Banks, 1904)**

Broad mite	Tarsonemidae	Trombidiformes

Ghosh et al. (2010)

21 polymorphic markers

$(GT)_{17}$, $(TG)_{12}$, $(GTT)_{8,}$ $(CTAT)_{18}$, $(GATA)_{17}GA$, $(CT)_{14}$, $(AG)_{21}$, $(AG)_{12}$, $(AG)_{23}$, $(CT)_{32}$, $(AG)_{16}$, $(GA)_{32}$, $(TAT)_6$, $(GA)_6$, $(CAA)_4$, $(CAA)_7$, $(CTT)_5$, $(TTA)_4$,

$(GAA)_5$, $(CTT)_4$, $(AAG)_4$		
14. ***Bryobia praetiosa*** **(Koch, 1836)**		
Clover mite	Tetranychidae	Trombidiformes
Weeks and Breeuwer (2001) 13 microsatellites tested. 3 amplified and were highly polymorphic $(AG)_{21}$, $(AG)_{24}$, $(AG)_{30}$		
15. ***Panonynchus citrii*** **(McGregor, 1916)**		
Spider mite, citrus red mite	Tetranychidae	Trombidiformes
Osakabe et al. (2000) 2 microsatellites were used in the study. Both highly polymrphic $(CT)_8(CA)_4CT$, $(CT)_4$ Sun et al. (2014) 15 microsatellites isolated $(ATC)_6$, $(TC)_6$, $(TC)_7$, $(TG)_7$, $(GGT)_6$, $(GGT)_7$, $(TC)_8$, $(GA)_6$, $(CAA)_6$, $(AG)_6$, $(AG)_7$, $(CT)_7$, $(GTT)_7$, $(TTA)_6$, $(ACC)_6$		
16. ***Tetranychus kanzawai*** **(Kishida, 1927)**		
Kanzawa spider mite	Tetranychidae	Trombidiformes
Nishimura et al. (2003) 7 microsatellites used $(AG)_{29}$ (63-97 bp), $(AG)_{18}$ (175-223 bp), $(AG)_{41}$ (168-214 bp), $(GA)_{23}$ (114-123 bp), $(GA)_{21}$ (216-327 bp) , $(CT)_{53}$ (159-229 bp), $(TC)_{39}$ (210-268)		
17. ***Tetranychus truncatus*** **(Ehara, 1956)**		
Red spider mite, cassava mite		
Hinomoto et al. (2009) 4 microsatellites isolated $(AG)_{13}$, $(CT)_{21}$, $(AG)_{23}$, $(CA)_6(CT)_8$		

18. *Tetranychus urticae* **(Koch, 1836)**		
Red spider mite		

Navajas et al. (1998, 2002)

5 microsatellites

$(GT)_n$, $(GT)_n$, $(GAT)_n$, $(GAT)_n$, $(TGA)_n$

Nishimura et al. (2003)

1 microsatellite

$(TC)_n$

(Uesugi and Osakabe, 2007)

9 microsatellites

$(CA)_n$, $(TC)_n$, $(GT)_n$, $(CT)_n$, $(CT)_n$, $(CA)_n$, $(GT)_n$, $(TG)_n$, $(GA)_n$,

Sun et al. (2012)

150 microsatellites were cloned, most were discarded because of short repetitive pattern or failure to amplify. 2 polymorphic clones were used: TECI104 (214 bp) TEC108 (110 bp).

$(GT)_6$, $(CT)_6$

Saune et al. (2015)

14 polymorphic microsatellites identified- TulS14, TulS16, TulS17, TulS19, TulS20, TulS22, TulS23, TulS24, TulS35, TulS38, TulS39, TulS41, TulS42, TulS43

$(ATG)_7$, $(CAT)_{10}$, $(ATG)_6$, $(TG)_6$, $(TTG)_6$, $(TG)_6$, $(TAA)_6$, $(GA)_7$, $(TG)_8$, $(CAA)_6$, $(AGC)_6$, $(CAT)_6$, $(ATC)_5$, $(GAT)_5$

Hada and Hinomoto (2016)

4 microsatellites used

$(CA)_7$, $(TC)_{13}$, $(CT)_8$, $(CT)_9$

Anguilar-Fernollosa et al. (2012)

7 locus-specific microsatellites used

$(CA)_n$ [276 bp), $(GT)_n$ [164 bp], $(TC)_n$ [205 bp], $(CT)_n$ [149 bp], $(CT)_n$ [296 bp], $(CA)_n$ [111 bp], $(TC)_n$ [210-268 bp]

Sabater-Muñoz et al. (2012)

7 microsatellites

$(CAC)_n$, $(CTT)_n$, $(AGA)_n$, $(GT)_n$, $(ACC)_n$, $(GAA)_n$		
Carbonnelle et al. (2007)		
5 microsatellites		
$(GT)_{30}$, $GT)_{29}$, $(GAT)_7$, $(GAT)_9$, $(TGA)_8$		
Li et al. (2009)		
3 microsatellites used		
$(GT)_{30,}$ $(GT)_{29,}$ $(TGA)_8$		
19. ***Paratarsotomus macropalpis*** **(Banks, 1916)**		
Water mite	Anystidae/Hygrobatidae	Trombidiformes
Asadi et al. (2008)		
13 microsatellites		
$(GT)_{12}(GA)_5GG(GA)_5$, $(TG)_{12}$, $(CT)_{13}$, $(TC)_{24}$, $(TG)_{23}$, $(CT)_6CG(CT)_4(CA)_{11}$, $(GA)_{36}$, $(AC)_{22}$, $(GT)_{15}(GA)_{24}$, $(GA)_{24}$, $(CT)_9$, $(AG)_9$, $(GA)_{24}$		
20. ***Leptotrombidium deliense*** **(Walch, 1922)**		
Scrub typhus mite	Trombiculidae	Trombidiformes
Chaisiri, 2016		
11 microsatellites developed.		
(GCT)n, (AAC)n, (AGC)n, (AAC)n, (GTTT)n, (AAG)n, (GTT)n, (ATC)n, (GAT)n, (ATT)n, (AAT)n		

Table 4.4 – Representative list of microsatellites developed for mite species. In the top part of the list is the only example of microsatellites developed without selection for predetermined motifs, called unbiased. All microsatellites selected for certain motifs are called biased microsatellites. The beginning of list records perfect microsatellites, whereas the rest of the list itemizes imperfect microsatellites. For perfect microsatellites, the motif and its reverse complement is given. For each motif, the number of repeats is enumerated, as far as known, plus the length of the microsatellite in base pairs.

4.5 Limitations of microsatellite analysis

One of the inherent challenges associated with the SSR markers is the slippage during the polymerase chain reactions. Under normal circumstances, slippage replications occur outside the polymerase chain reactions. The role of the PCR is to amplify amplicon length so that researchers can observe the allelic variations. In some cases, the slippage may occur during the polymerase chain reactions. This type of reaction may crease 'stutter bands' that differ from the main product by a series of repeated units. In other words, the 'stutter bands' may abnormally increase the repeat length of the microsatellite profiles. The stutter bands may also create ladder bands especially in the case of missing prominent bands in the ladder. The main problem associated with the stutter bands is quasi scoring. This may affect the outcomes because it creates two sets of results which may be conflicting. Quasi scoring denies the SSR marker the accuracy it requires. Moreover, stuttering may also cause ambiguity in the interpretation of the SSR marker because it creates long stretches of short repeated units. If one is not keen, he or she may mistake the long stretch for a different sequence or pattern.

In some cases, a character derived in two species may look similar not because they come from the same ancestry but because the similarity has been caused by convergence, reversion or parallelism. This phenomenon is known as homoplasy and can potentially interfere with the accuracy of the final outcomes. Homoplasy creates a scenario where copies of the locus are identical in state but different in descent. Homoplasy is a potential threat to accuracy because it can lead to mistaken identities. A person can easily associate one characteristic with two species even without considering their genetic origins. In the microsatellite analysis, homoplasy may occur when two bands are similar in size but differ in sequence. To determine the effects of homoplasy, an experiment was conducted in which four *Apis* species were tested. The four different species that were studied in the experiment includes *A. mellifera*, *A. lingustica, A. scutellata, and A. capensis*. The researchers found two sets of identical eletromorphs. One set of identical eletromorphs was associated with *A. millifera* and *A. lingustica*, the other set was linked to *A. scutella* and *A. capensis*, respectively. In this study, the two bands

obtained from *A. scutellata* and *A. capensis* were similar in size but different in sequence.

One of the negative effects of homoplasy is enhanced ambiguity in studying the genetic characteristics of the different species. For instance, if two species end up with bands of the same size but different sequence, observers may find difficulties in telling the original SSR locus. The enhanced ambiguity may also lead to underestimation of genetic variations in a population. Ambiguous outcomes are not required in genetic analysis because it affects the validity of the outcomes. Any wrong conclusion in a DNA analysis may have far reaching consequence depending on the application of the results.

4.6 How many microsatellites are necessary for mite species to distinguish neighbouring populations?

The standard proportion of the microsatellites needed to distinguish mite species from neighbouring populations may vary depending on the type of the amplification. The amplification process only requires a small quantity of DNA to provide the required PCR products. In most cases, the standard quantity for a genome scan is 5 µg of each DNA sample. For example, a 5 µg of *Tetranychus urticae* and another 5 µg of *Sarcoptes* mites can be used to distinguish the two species. In most DNA, experiments there is no restriction over the amount of species that researchers should supply. What is usually restricted is the format in which the species should be presented. The recommended format is a standard concentration presented in a microtiter plate. Besides, the use of microsatellites gives researchers an advantage because PCR process only requires a limited quantity of the DNA sample.

The use of microsatellites in the analysis of DNA obtained from mites has highlighted various genetic characteristics of the population. One of the observations is the impact of geographical location on the genetic characteristics of mites. In studies where the same species of mites were obtained from different geographical locations, researchers identified significant allelic variations in the genetic characteristics. In other words, same species of mites obtained from

England, Germany and the Netherlands are likely to have different genetic characteristics. Another study has also identified the impact of hosts on the genetic characteristics of mites.

4.7 Conclusions

This review shows that the microsatellites developed in this work are only the second based on whole genome sequencing in Acari. While the first study using whole genome sequencing investigated microsatellites up to pentanucleotide repeats, the current work searched for microsatellites up to decanucleotide repeats in a whole genome and reports these for the first time.

4.8 References

Aguilar-Fenollosa E, Pina T, Gomez-Martinez MA, Hurtado MA and Jacas JA (2012) Does host adaptation of *Tetranychus urticae* populations in clementine orchards with a *Festuca arundinacea* cover contribute to a better natural regulation of this pest mite? *Entomologia Experimentalis et Applicata* 144: 181-190.

Andre T (2019) Microsatellite instability (MSI): best predictor of effectiveness of immune checkpoint inhibitors (ICKi)? Focus on MSI by Bulletin du Cancer. *Bulletin du Cancer* 106: 91-93.

Asadi M, Higaki T, Hinomoto N, Saboori A and Naghavi MR (2009) Isolation and characterization of polymorphic microsatellite loci from the water mite *Hygrobates fluviatilis* (Acari: Hydrachnidia: Hygrobatidae). *Molecular Ecology Resources* 9: 793-799.

Bagshaw ATM (2017) Functional mechanisms of microsatellite DNA in eukaryotic genomes. *Genome Biology and Evolution* 9: 2428-2443.

Bailly X, Migeon A and Navajas M (2004) Analysis of microsatellite variation in the spider mite pest *Tetranychus turkestani* (Acari: Tetranychidae) reveals population genetic structure and raises questions about related ecological factors. *Biological Journal of the Linnean Society* 82: 69–78.

Baron S, van der Merwe NA and Maritz-Olivier C (2018) The genetic relationship between *R. microplus* and *R. decoloratus* ticks in South Africa and their population structure. *Molecular Phylogenetics and Evolution* 129: 60-69.

Beaurepaire AL, Krieger KJ and Moritz RFA (2017) Seasonal cycle of inbreeding and recombination of the parasitic mite *Varroa destructor* in honeybee colonies and its implications for the selection of acaricide resistance. *Infection, Genetics and Evolution* 50: 49-54.

Beaurepaire AL, Ellis JD, Krieger KJ and Moritz RFA (2019) Association of *Varroa destructor* females in multiply infested cells of the honeybee *Apis mellifera*. *Insect Science* 26: 128-134.

Butler JM (2005) *Forensic DNA Typing: Biology and Technology behind STR Markers*. 2nd edn. Academic Press, London.

Carbonnelle S, Hance T, Migeon A, Baret P, Cros-Arteil S and Navajas M (2017) Microsatellite markers reveal spatial genetic structure of *Tetranychus urticae* (Acari: Tetranychidae) populations along a latitudinal gradient in Europe. *Experimental and Applied Acarology* 41: 225-241.

Carew ME, Goodisman MAD and Hoffman AA (2004) Species status and population genetic structure of grapevine eriophyoid mites. *Entomologia Experimentalis et Applicata* 111: 87-96.

Chaisiri K (2016) *Molecular ecology of chigger mites (Acari: Trombiculidae) and associated bacteria in Thailand*. University of Liverpool, Liverpool

Evans JD (2000) Microsatellite loci in the honey bee parasitic mite *Varroa jacobsoni*. *Molecular Ecology* 9: 1436-1438.

Evans LM, Dawson DA, Wall R, Burke T and Stevens JR (2003) Isolation of *Psoroptes* scab mite microsatellite markers. *Molecular Ecology Notes* 3: 420-424.

Ge C, Sun JT, Cui YN and Hong XY (2013) Rapid development of 36 polymorphic microsatellite markers for *Tetranychus truncatus* by transferring from *Tetranychus urticae*. *Experimental and Applied Acarology* 61: 195–212.

Ghosh A, Sharmin S, Islam S, Pahloan MU, Islam S and Khan H (2010) SSR markers linked to mite (P*olyphagotarsonemus latus* Banks) resistance in Jute (*Corchorus olitorius* L.). *Czech Journal of Genetics and Plant Breeding* 46: 64-74.

Goldstein DB and Schlötterer C (eds) (1999) *Microsatellites: Evolution and Applications*. Oxford University Press, Oxford; pp 368.

Goodwin W (ed) (2016) *Forensic DNA Typing Protocols*, vol 1420. Methods in Molecular Biology, 2nd edn. Humana Press, New York; pp 310.

Guichoux E, Lagache L, Wagner S, Chaumeil P, Léger P, Lepais O, Lepoittevin C, Malausa T, Revardel E, Salin F and Petit RJ (2011) Current trends in microsatellite genotyping. *Molecular Ecology Resources* 11: 591-611.

Hada H, Hinomoto N and Gotoh T (2016) Genetic structure of *Tetranychus urticae* (Acari: Tetranychidae) populations under acaricide selection pressure assessed using microsatellite markers. *Systematic and Applied Acarology* 21: 878-881.

Hill MP, Hoffmann AA, Umina PA, Cheng X and Miller AD (2016) Genetic analysis along an invasion pathway reveals endemic cryptic taxa, but a single species with little population structure in the introduced range. *Diversity and Distributions* 22: 57-72.

Hinomoto N and Maeda T (2005) Isolation of microsatellite markers in *Neoseiulus womersleyi* Schicha (Acari: Phytoseiidae). *Journal of the Acarological Society of Japan* 14: 25-30.

Hinomoto N, Higaki T, Osakabe M and Takafuji A (2009) Development and evaluation of microsatellite markers in *Tetranychus truncatus* Ehara (Acari: Tetranychidae). *Journal of the Acarological Society of Japan* 18: 91-98.

Hinomoto N, Todokoro Y and Higaki T (2011) Population structure of the predatory mite *Neoseiulus womersleyi* in a tea field based on an analysis of microsatellite DNA markers. *Experimental and Applied Acarology* 53: 1-15.

Huber K, Jacquet S, Rivallan R, Adakal H, Vachiery N, Risterucci AM and Chevillon C (2019) Low effective population sizes in *Amblyomma variegatum*, the tropical bont tick. *Ticks and Tick-Borne Diseases* 10: 93-99.

Kantartzi SK (ed) (2013) *Microsatellites: Methods and Protocols*. Methods in Molecular Biology, vol 1006. Humana Press, New York; pp 352.

Li T, Chen X-L and Hong X-Y (2009) Population genetic structure of *Tetranychus urticae* and its sibling species *Tetranychus cinnabaribus* (Acari: Tetranychidae) in China as inferred from microsatellite data. *Annals of the Entomological Society of America* 102: 674-683.

Miller AD, Umina PA, Weeks AR and Hoffman AA (2012) Population genetics of the wheat curl mite (*Aceria tosichella* Keifer) in Australia: Implications for the management of wheat pathogens. *Bulletin of Entomological Research* 102: 199–212.

Navajas M, Perrot-Minnot MJ, Lagnel J, Migeon A, Bourse T and Cornuet JM (2002) Genetic structure of a greenhouse population of the spider mite *Tetranychus urticae*: Spatio-temporal analysis with microsatellite markers. *Insect Molecular Biology* 11: 157-165.

Navajas MJ, Thistlewood HMA, Lagnel J and Hughes C (1998) Microsatellite sequences are under-represented in two mite genomes. *Insect Molecular Biology* 7: 249-256.

Naz S, Chaudhry FR, Rizvi DA and Ismail M (2018) Genetic characterization of *Sarcoptes scabiei* var. *hominis* from scabies patients in Pakistan. *Tropical Biomedicine* 35: 796-803.

Nishimura S, Hinomoto N and Takafuji A (2003) Isolation, characterization, inheritance and linkage of microsatellite markers in *Tetranychus kanzawai* (Acari : Tetranychidae). *Experimental and Applied Acarology* 31: 93-103.

Osakabe M, Hinomoto N, Toda S, Komazaki S and Goka K (2000) Molecular cloning and characterization of a microsatellite locus found in an RAPD marker of a spider mite, *Panonychus citri* (Acari: Tetranychidae). *Experimental and Applied Acarology* 24: 385–395.

Paulauskas A, Galdikas M, Galdikaite-Braziene E, Stanko M, Kahl O, Karbowiak G and Radzijevskaja J (2018) Microsatellite-based genetic diversity of *Dermacentor reticulatus* in Europe. *Infection, Genetics and Evolution* 66: 200-209.

Rasolofoarivao H, Clemencet J, Speck A, Raveloson-Ravaomanarivo LH, Reynaud B and Delatte H (2017) Genetic diversity of *Varroa destructor* parasitizing *Apis mellifera* unicolor in Madagascar. *Apidologie* 48: 648-656.

Sabater-Muñoz B, Pascual-Ruiz S, Gómez-Martínez MA, Jacas JA and Hurtado MA (2012) Isolation and characterization of polymorphic microsatellite markers in *Tetranychus urticae* and cross amplification in other Tetranychidae and Phytoseiidae species of economical importance. *Experimental and Applied Acarology* 57: 37-51.

Sauné L, Auger P, Migeon A, Longueville JE, Fellous S and Navajas M (2015) Isolation, characterization and PCR multiplexing of microsatellite loci for a mite crop pest, *Tetranychus urticae* (Acari: Tetranychidae). *BMC Research Notes* 8: 247-256.

Schausberger P, Patiño-Ruiz JD, Osakabe M, Murata Y, Sugimoto N, Uesugi R and Walzer A (2016) Ultimate drivers and proximate correlates of polyandry in predatory mites. *PLoS ONE* 11: e0154355.

Shi P, Cao L-J, Gong Y-J, Ma L, Song W, Chen J-C, Hoffmann AA and Wei S-J (2019) Independently evolved and gene flow-accelerated pesticide resistance in two-spotted spider mites. *Ecology and Evolution* 9: 2206-2219.

Solignac M, Vautrin D, Pizzo A, Navajas M, Le Conte Y and Cornuet JM (2003) Characterization of microsatellite markers for the apicultural pest *Varroa destructor* (Acari: Varroidae) and its relatives. *Molecular Ecology Notes* 3: 556-559.

Sun J-T, Lian C, Navajas M and Hong XY (2012) Microsatellites reveal a strong subdivision of genetic structure in Chinese populations of the mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *BMC Genetics* 13: e8.

Sun J-T, Kong L-W, Wang M-M, Jin P-Y and Hong X-Y (2014) Development and characterization of novel EST-microsatellites for the citrus red mite, *Panonychus citri* (Acari: Tetranychidae). *Systematic and Applied Acarology* 19: 499-505.

Uesugi R and Osakabe MH (2007) Isolation and characterization of microsatellite loci in the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae): Primer note. *Molecular Ecology Notes* 7: 290 - 292.

van Schaik J, Dekeukeleire D and (Kerth G (2015) Host and parasite life history interplay to yield divergent population genetic structures in two ectoparasites living on the same bat species. *Molecular Ecology* 24: 2324-2335.

Walton SF, Currie BJ and Kemp DJ (1997) A DNA fingerprinting system for the ectoparasite *Sarcoptes scabiei*. *Molecular and Biochemical Parasitology* 65: 187-196.

Weeks AR and Breeuwer JAJ (2001) *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. *Proceedings of the Royal Society Biological Sciences Series B* 268: 2245-2251.

5 Bead beating of mites: Extracting DNA for forensic analysis

5.1 Introduction

Beat beating is considered the most effective technique of disrupting biological samples containing DNA of mites or insects. The process is achieved through rapid agitation of samples using a grinding media that consists of balls or beads in a bead beater. A beat beater acts as a shaker to break the sample in the homogenization vessel. The bead beaters are developed in a manner that allows homogenisation of samples in tubes, microwell plates, or vials with the grinding media. Evidently, the media is made up of steel, ceramic, or glass with zirconium or silica. The samples are processed cryogenically or at room temperature regardless of the presence of a solvent or buffer. The procedure used leads to the extraction of high quality and large DNA sample for molecular analysis, thus identifying the process as the most crucial for extracting nucleic molecules from insects and mites.

The beat beating process is carried out in a homogenizer such as MiniG and the Geno/Grinder that processes samples in many formats. High throughput homogenizers are essential for processing mite or insect samples in deep well plates of up to 50 µl. The homogenizers consist of a linear motion that directs kinetic energy of beads or balls towards the sample. The linear motion bead beaters can homogenize event resilient samples such seeds. The bead beat technique is applied in the extraction of mite or insect DNA since it is an effective technique of breaking down most samples. Bead beater also reduces cross-contamination when processing many samples simultaneously since it lacks direct contact with the samples. Evidently, this technique is well distinguished from other DNA extraction techniques such as the handheld rotor-stators. This scenario led to the formulation of different bead beaters to enhance the quality of the sample generated within a short time.

There are different categories of instruments used in the bead beating DNA extraction process based on the amount and number of samples processed, agitation speed, and physical motion. The classification of bead beating instruments is also associated with the character of the sample used and the homogenizer, which includes size, shape, density, and the efficiency of the extraction process. In all the varieties of bead beating devices, the vial, plate, or tube is shaken so that the beads

or balls can influence the disruption of the sample. The bead beaters are effective based on the number of samples used, the extraction protocol, speed, and motion.

Vortexers are the most commonly used bead beaters since they are the simplest bead beaters in application to date. They function by swirling the grinding media and the sample in a manner that enhances disruption of the mites. Vortexers are the less effective bead beaters used in the extraction of DNA. For extraction of large DNA, shaking mills and dental amalgamators are used in which they oscillate to form a figure-eight motion that allows grinding and crushing of the DNA samples. Resilient samples are homogenized using high throughput homogenizers in a linear motion bead beater. The vortexers achieves maximum processing of DNA samples from microorganisms such as mites and insects rather than other organisms. Thus, the vortexers are the widely used DNA extraction channels for insects and mites.

DNA isolation methods for Acari

Homogenization		
Beat beading	ticks	(Ammazzalorso et al., 2015)
	ticks	(Kato and Mayer, 2013)
	ticks	(Crowder et al., 2010)
	ticks	(Halos et al., 2004)
	Demodex canis	(Toops et al., 2010)
DNA isolation of oribatid mites		
Silica membrane Macherey-Nagel, Qiagen peqlab	adequate	(Lienhard and Schaeffer, 2019)
Protein precipitation Promega	not-adequate	
CTAB and phase separation Sigma	not-adequate	
CTAB and silica Omega Bio-tek	not-adequate	
Chelex Bio-Rad	moderate	

Table 5.1 – Examples of DNA isolation methods using beat beading and various DNA purification methods employed for Acari.

Single and multitube vortexers are used to disrupt microorganism in a sample but less effective to homogenize solid tissues. On that note, the homogenizers are essential in laboratories with financial constraints and when processing small samples of microorganisms. Vortex disrupts the cells through adding a suspension of mites and the grinding media in a snap cap tube and holding it in a vortex mixture for a short while. Nonetheless, the technique is usually less effective than other dedicated bead beaters offered commercially, which are modified to operate as a bead beater. The mostly used vortexer include the high throughput processing multitube vortexer that accommodates many racks of microwell plates or tubes and the pulsing vortexer mixer that processes approximately ten samples in the microcentrifuge tubes.

The bead beating acts as a homogenization process, which is used to break up the cell membrane present in samples to release the genetic material and proteins present in the cell. Mite or insect specimens stored in extremely cold conditions, usually at more than - 80 °C, are usually scraped using a microtip and incubated using the appropriate medium at body temperature overnight. The samples are then placed in a tube with grinding beads in which it is subjected to high energy mixing. Materials, apparatus, and equipment used in the DNA extraction process include 1ml Tris-ethyl diamine tetra acetate (TE) buffer, eppendorf tube, and microcentrifuge among others. The TE buffer is made up of 10 mM Tris-HCL and 1 mM EDTA at a pH of 8.0. Other materials are also applied with some minor modifications to favour the activity of the GES method. A GES solution is usually prepared for the bead beating technique, which entails the inclusion of guanidium thiocyanate, EDTA at pH 8.0, distilled water, and sarkosyl. The Beads Cell Disrupter Micro Smash is usually used as a bead-beating disruption apparatus.

The extraction of genomic DNA acts as the first step in the analysis and characterisation of DNA for various uses in molecular biology. In the extraction of macromolecules, there are three basic steps, which involve cell lysis, separation of the macromolecules from other cell components, and purification of the extracted DNA from the reagents used during the extraction process and other cell fragments. The cells are collected and sample for the extraction process and the cells are broken

using beads. The cells of insects and mites can be disrupted using detergents and enzymes, which may entail the use of detergents, sodium dodecyl sulphate (SDS), or beads to lysis. In addition, the sonication process can also be used to disrupt the cell wall of the samples of insects or mites used. In addition, the bead beating process is usually carried out using customised jars for DNA processing, deep well plates, vials, or microfuge tubes. After the cells are broken, the lipids, RNA, and proteins are removed through the addition of detergents, RNases, and proteases respectively. DNA is then purified from detergents using phenol chloroform isoamyl alcohol, ethanol, or mini-column purification. The extracted DNA through bead beating is used in genetic engineering, molecular and medical diagnosis, production and manufacture of industrial products, as well as cloning and molecular analysis. The DNA from mites is the first step in the production of genetically engineered animals. It involves the editing of a single gene to transplant of genes to other organisms.

The bead beating process enables the development of cloned mites that facilitates production of medicinal compounds for the treatment of certain illnesses such as the allergic reactions caused by *Dermatophagoides farinae* and *D. pteronyssinus*. DNA extracted from the mites and insects can be used to analyse and characterise the diseases that affect the organisms. The chiggers of Asia transmit rickettsia while those of North America cannot. The house mouse mite, commonly referred to as *Liponyssoides sanguineus* transmits rickettsiapox in North America, which is caused by *Rickettsia akari*. Most houses in the urban centres are the most affected by mites since the organism thrive comfortably in the enabling environment. The bead beating DNA extraction protocol in mites as well as in insects is an important factor in the isolation of genomic DNA from the organisms.

5.2 Materials and Methods

Comparison of bead type and size for the genomic DNA extraction of various mite species; in short, beating DNA out of mites.

The comparison is done with four mite species:

1. *Tyrolichus casei* as a future model lab or food mite

2. *Dermatophagoides pteronyssinus* as a model dust mite
3. *Tyrophagus putrescentiae* as a second model dust mite
4. *Archegozetes longisetosus* as a model soil mite

All the experiments have been done in triplet (three times) to allow calculation of an average and a standard deviation.

The following beads will be compared

1. Silica (glass) beads 0.1 mm
2. Silica (glass) beads 0.5 mm
3. Silica (glass) beads 1.0 mm
4. Zirconia (ceramic) beads 0.1 mm
5. Zirconia (ceramic) beads 0.5 mm
6. Zirconia (ceramic) beads 1.0 mm
7. Zirconia (ceramic) beads 1.5 mm
8. Zirconia (ceramic) beads 3.0 mm
9. Steel beads 2.8 mm
10. Carbide beads 0.28 mm
11. Carbide beads 0.70 mm
12. Carbide beads 3.0 mm
13. Garnet beads 0.15 mm

Silica, zirconia, and steel: Benchmark Scientific through Progen Scientific
Carbide and garnet: Mo Bio through Cambio
Carbide 3 mm: Qiagen
Zirconia: zirconium oxide
Glass beads: acid washed
Zirconium beads: TriplePure, acid washed, heat-treated
Carbide: tungsten carbide
Tungsten carbide reacts chemically with lysis buffers used for RNA extraction.
Garnet: iron-aluminum silicate

The comparison of the beads will be based on

- size 0.1 – 3.0 mm
- density (Toops et al., 2010) to a single none-bead method:
- Meta-G-Nome™ DNA Isolation Kit

which relies completely on chemical and enzymatic lysis; a kit developed for genomic DNA extraction for next generation sequencing applications.

The quantity and quality of the extracted DNA will be measured

• yield	A260	A260-A320
• purity	A260/280	(A260-A320)/(A280-A320)
• A260/230		(A260-A320)/(A230-A320)
• turbidity	A320	

5.3 Results

The effects of bead size, 0.1 – 3.0 mm, the effects of bead density, glass 2.5 g/cm3, zirconia 5.5 g/cm3, steel 7.9 g/cm3, carbide 14.9 g/cm3, and garnet 3.9 g/cm3, the effects of shape, round versus sharp edges on the recovers of DNA from four different species of mites were investigate, Figure 5. 1 and 5.2.

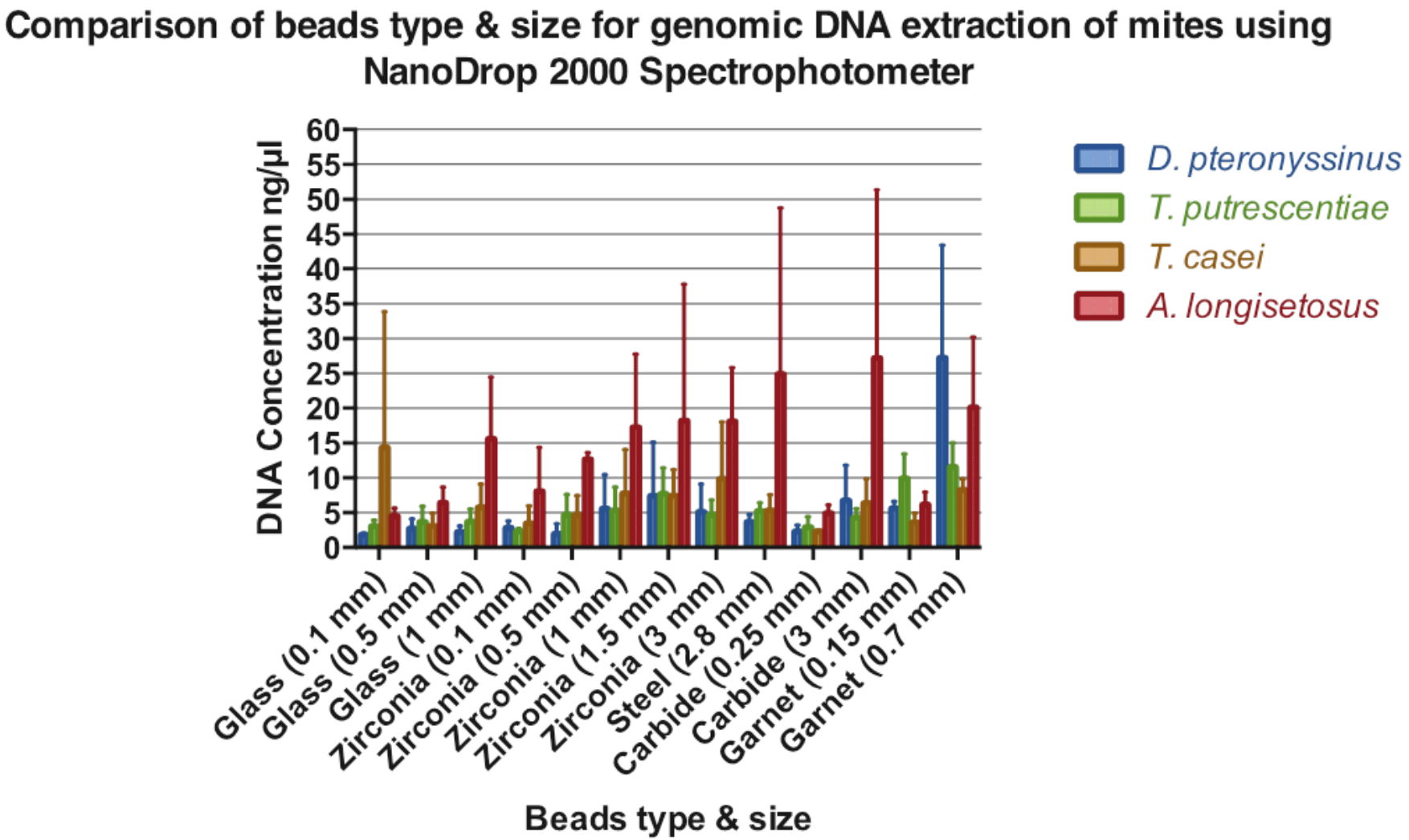


Figure 5.1 – Error bars represent standard deviation of three independent beating and DNA isolation experiments.

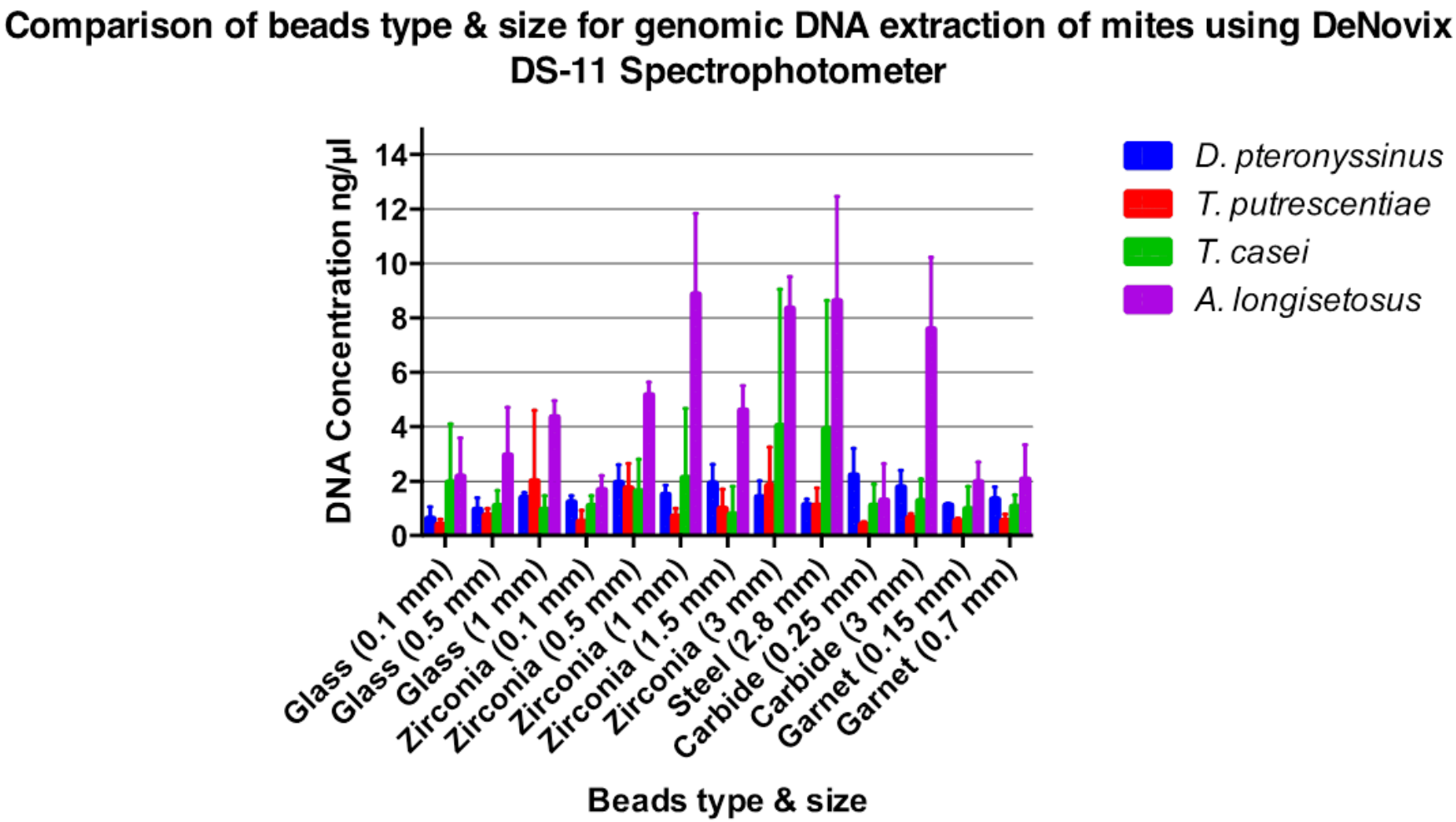


Figure 5.2 – Error bars represent standard deviation of three independent beating and DNA isolation experiments.

5.4 Discussion

5.4.1 DNA quantification

The high amount of DNA of the first set of experiments was astonishing, Figure 5.1. When these DNA preparations were analysed by a tape station for library preparation for whole genome sequencing, the amount of DNA was around a quarter of the DNA analysis with the industry standard of a NanoDrop 2000 spectrophotometer. This led us to prepare reference standard that were analysed with the NanoDrop, Qubit, and a tape station, which confirmed that the average readings of a Nanodrop and a Qubit much too high. This was confirmed by several genome sequencing facilities. A DeNovix DS-11 spectrophotometer was acquired which, uniquely, has a self-calibration mechanism. The lack of calibration of the NanoDrop and Qubit instruments might be a major reason for their excessive high readings.

Reference samples gave the same quantification with a DeNovix DS-11 spectrophotometer as with a tape station.

The experiments were repeated with a DNA quantification with a a DeNovix DS-11 spectrophotometer, Figure 2.

5.4.2 Bead size

It was expected that the beat size correlates with the size of the sample. Small samples like bacteria should use the smallest beat sizes, while large samples like entire mites should be beast broken up with the largest beat sizes. Although this trend is visible in some of the samples, the correlation is statistically not significant. The conclusion is that beat size is not a crucial factor for the extraction of DNA from mites.

5.4.3 Bead density

It was expected that beat density is important for samples that carry a chitin-based exoskeleton like mites. The results do not confirm that. Steel has three times the density of glass, and carbide has twice the density of steel. Although the amount of DNA extracted with steal and carbide beads is higher than that extracted with glass

beads, the correlation is statistically not significant. The conclusion is that the higher density of steel and carbide beads is desirable for the extraction of DNA from mites.

5.4.4 Bead shape

The same argument that is put forward for using a higher density of beats for whole organism with a chitin-based exoskeleton is also employed for promoting the razor-sharp ends of carbide and garnet particles over the smooth round surface of glass, zirconium, and steel beads. Again, the results do not confirm that. There is no statistically not significant correlation. The conclusion is that beat size is not a crucial factor for the extraction of DNA from mites.

5.4.5 Sample type

The results show that the most important factor for maximizing the quantity of DNA to be extracted is the mite species itself. This would suggest a correlation with the estimated genome size of the mite species, however, no statistically significant correlation could be found. It was assumed that 'roughest' habitat for the four mite species in this experiment would be soil; and therefore the soil mite, *Archegozetes longisetosus,* would be the toughest to crack and extract, and the cheese mite, *Tyrolichus casei, the easiest*. This is not the case. Repeatedly, the highest amounts of DNA could be extracted from *A. longisetosus.*

The bead *beating* process acts as an effective process used for the disruption of numerous biological samples, especially when extracting mite or an insect's DNA. The extraction process is achieved through agitating the sample with a grinding medium in a bead beater in which the samples are processed in presence or absence of buffer at room temperature. High throughput homogenizers such as MiniG® and The Geno/Grinder® are used for processing samples in well plates. Different samples have varying resiliency that facilitates homogenization through bead beating. Balancing sample masses, the material of the grinding media, and vessel volume plays a crucial role in shaping the bead beating process. It is believed that developing an optimum protocol for reaching the desired endpoint enhances the

success of the methods used. Other factors associated with the success of the beat beading process include processing parameters, cryogenic homogenization, and the grinding media. High-density grinding media such as stainless steel and zirconia are effective than silica beads.

Nevertheless, some materials may lead to the generation of heat during grinding.

The grinding media is usually divided into ceramic satellites, grinding balls, and grinding resins. The grinding beads fall within a specific size in which the grinding balls are spherical and precision with a normal diameter. Bead beats with small length disrupt microorganisms such as bacteria and cells from mites while larger beads homogenize the presented samples. In addition, the stainless-steel balls may oxidize in the presence of phenolic compounds, which favour interference with subsequent processing, cylindrical, and ceramic satellites. Grinding resins are sharp, irregularly shaped composites, such as garnet, that can also be effective at shredding resilient. Nevertheless, the inherent properties and composition of the grinding media affect sample homogenization. Zirconium, silica, and stainless steel are widely used as grinding media.

The bead beater disrupts most of the cells in a short while. In addition, the homogenization process involves cell cracking action rather than high shear forces. After homogenization, cell membranes appear to be intact when viewed under a microscope. Therefore, one should rely on assay methods that measure intracellular constituents such as the Polyacrylamide Gel Electrophoresis (PAGE) if the goal is to isolate intact intracellular organelles. The bead beating process is the most effective that favours improving the yield of intact organelles in which it homogenizes for a shorter while and causes approximately 70 % maximum cell disruption. The bead beats also facilitate the extraction of large amounts of DNA due to proper temperature control and bead size selection. The standard diameter for beads is between 0.1 mm to 2.5 mm. Nevertheless, while glass bead media is most commonly used, the denser bead media is also present for availability for tough materials. In addition, the bead beating process is enhanced by proper temperature control mechanism since the homogenate is warmed after three minutes. Cooling is

sometimes essential when isolating proteins, membranes, or organelles. According to Desloire et al. (2006), temperature control is usually not a necessity when isolating nucleic acids in an aggressive extraction media that is made up of phenol-chloroform, guanidinium salts, or detergents.

The bead beating technique is a common laboratory mechanical method for the disruption of samples containing nucleic acids using tiny steel or glass beads in which the sample is suspended in the media. Shaking the vessels during the reaction may subject the bead mix and the sample to high-level agitation. In addition, the beads collide with the cellular sample, thereby cracking open the cell to release intercellular components. During homogenization, mechanical shear is moderated thereby leading to excellent subcellular and membrane preparations. The bead beating technique offers an exclusive operational mechanism for most of the cellular material in a wide range from plant tissues, small organisms, animals, microorganism, and spores. The technique is advantageous compared to other mechanical cell disruption techniques. In fact, the methods lead to the disruption of small sample sizes and process many samples with little to no cross-contamination concerns.

On the other hand, the bead beating technique has several demerits that affect the quality of the extracted DNA for molecular purposes. Rapid advances in genetic studies have enhanced the use of molecular techniques in various genome identification mechanisms and molecular biology.

Adequate isolation and purification of mite and insect DNA is a critical step in ensuring the success of the techniques. The standard DNA extraction techniques developed for animal cells are not readily applied. Nonetheless, the most common techniques applied for the extraction of insects' genomic DNA entails the use of cell wall degrading enzyme as well as a physical grinding treatment, which is usually undertaken with liquid nitrogen. For DNA extraction, the last physical treatment is the common technique applied. It consists of freezing the cells with liquid nitrogen and grinding them with a mortar and a pestle. Moreover, numerous studies have portrayed excellence in the application of the mechanical bead-beating extraction procedure to isolate genomic DNA from mites. The technique has several

advantages over the traditional ones since it reduces time in which multiple sample extraction can take place.

5.5 Conclusions

Beat beading has become the method of choice for ticks (Acari: Parasitiformes). With the exception of *Demodex canis* (Acari: Trombidiformes), beat beading has not been investigated for mites other than ticks. Beat beading has been proven here to be an efficient method for the extraction of DNA from a wide variety of mite species. The importance of the bead characteristics on DNA recovery seems less than expected.

5.6 References

Ammazzalorso AD, Zolnik CP, Daniels TJ and Kolokotronis S-O (2015) To beat or not to beat a tick: comparison of DNA extraction methods for ticks (*Ixodes scapularis*). *PeerJ* 3: e1147.

Crowder CD, Rounds MA, Phillipson CA, Picuri JM, Matthews HE, Halverson J, Schutzer SE, Ecker DJ and Eshoo MW (2010) Extraction of total nucleic acids from ticks for the detection of bacterial and viral pathogens. *Journal of Medical Entomology* 47: 89-94.

Halos L, Jamal T, Vial L, Maillard R, Suau A, Le Menache A, Boulouis HJ and Vayssier-Taussat M (2004) Determination of an efficient and reliable method for DNA extraction from ticks. *Veterinary Research* 35: 709-713.

Kato CY and Mayer RT (2013) Cost-effective bead-based method for high-throughput homogenization of individual small arthropods. *Journal of Vector Borne Diseases* 50: 62-64.

Lienhard A and Schaeffer S (2019) Extracting the invisible: obtaining high quality DNA is a challenging task in small arthropods. *PeerJ* 7: e6753.

Toops E, Blagburn B, Lenaghan S, Kennis R, MacDonald J and Dykstra C (2010) Extraction and characterization of DNA from *Demodex canis*. *International Journal of Applied Research in Veterinary Medicine* 8: 31-43.

6 Genome analysis of *Dermatophagoides farinae* for repetitive sequences and the development of microsatellites

6.1 Introduction

House dust mites are a considerably large group of free-living organisms that inhabit houses, mattresses, beddings, and carpets. The major species of house dust mites include *Dermatophagoides farinae* and *D. pteronyssinus*, which play a crucial role in the development of asthma and allergic reactions.

House dust mites originate from parasites associated with mammals and birds, which are usually found in dwellings with dust. The mites bring about positive cutaneous reactions in individuals with sensitive or allergic conditions. The organisms are nearly distributed in all dusty houses and bird nests. They are well distributed in the tropics as well as temperate areas in which their number increases during summer and lowers in the winter. Propagation of the organisms is affected by the relative humidity and temperature. High concentrations of the mites are found in brick houses compared to wooden houses. The organisms usually inhabit mattresses and carpets since they offer an enabling environment for their multiplication.

D. farinae are cosmopolitan organisms found in most habitats across the globe especially in North America. The mites are small size, translucent organisms that are invisible with naked eyes. Evidently, a standard *D. farinae* measures 0.2-0.3 millimetres in length. The female and male measures approximately 420 microns in length, whereas their width differs from 245 to 320 microns (Males and females have different structures, whereby the males are sclerotized with enlarged legs III and I. The males also have a pair of suckers used to grasp the females during copulation. The suckers are located on the ventra I posterior idiosoma. In addition, the males have aedeagus between apodemes of leg IV while the female genital openings are located in the *bursa copulatrix*.

The life cycle of *D. farinae* is characterised by egg, larva, protonymph, tritonymph, and imago. According to Colloff (2018), the mites require approximately one month for a complete life cycle based on the humidity and temperature. The females usually live for 60 days, whereby a male attaches onto the female after it reaches the adult stage. *D. farinae* is affected by relative humidity, whereby it stops growing or dies at a humidity of 60 % or lower.

Scientists developed methods for separating the organisms from other components of their habitat such as carpets, chairs, and mattresses through vacuum cleaning. The mites can be identified by placing the dust on water and examining it under 20 x magnifications or higher. *D. farinae* in the samples float on the water through the application of a sieving and floatation technique. For instance, a sample of dust is placed on a saturated sodium chloride solution and a few drops of the detergent are added. The suspension is the rinsed using a mesh sieve and stained with crystal violet to enable viewing under a stereo microscope. The mites are attracted to a white sheet of paper, which is examined over a lamp and identified by a cast of shadow.

D. farinae feeds on skin flakes from humans and some species of moulds. The organisms can be reared on a complex organic and chemically defined diet in which higher number of mites was produced on the organic diet after approximately a year. After culturing, the culture extracts are used for the treatment of several diseases such as skin disorders, immunotherapy, and researches. Evidently, diet of the organisms plays a crucial role in enhancing their culturing, growth, and survival.

D. farinae are microscopic organisms thriving in upholstered furniture, carpets, curtains, bedding, and mattresses. They feed on flakes of skin from people and pets in warm and humid regions. The mites also are linked to allergies and certain diseases such as dermatitis, asthma, and rhinitis. It is believed that allergic reactions associated with dust mites are characterised by sneezing, nasal congestion, post nasal drip, facial pressure, runny nose, itchy nose, roof of mouth or throat, pain, itchy red or watery eyes, and cough. The mechanism of the organisms to cause illnesses is caused by the unique, unstable, and partially understood genetic code.

The Asthma and Allergy Foundation of America showed that dust mites are the leading cause of asthma and allergy in most states. However, it is technically hard to eliminate all the organisms in a homestead. Their activity is reduced with chemicals or natural means to lower chances of developing allergies and related disorders. *D. farinae* can be controlled in a homestead through lowering temperature and humidity, cleaning the house regularly, washing soft furnishing and beddings using high temperatures, and sprinkling diatomaceous earth in the house. In

addition, the organisms can also be controlled through freezing, embracing minimalism, eliminating houseplants in the house, and spraying essential oils. This technique is an essential mechanism to overcome the multiplication of the organism, thus reducing cases of diseases linked to the mites.

Nevertheless, taxonomic classification helps in the identification of various species of dust mites, which plays a crucial role in the development of chemicals to overcome or kill them. The classification also enabled the construction of important and reliable keys for mapping the genetic code of the organism. According to An et al. (2013), the taxa enhance reproducible identification of the organism for ecological and environmental purpose. Advances of current keys to dust mites and interactive computer keys enhance understanding of the behaviours of the organisms. Representative articles on key biological features of *D. farinae* are listed in Table 6.1.

Biological features of *Dermatophagoides farinae*

Heteromorphic males	Solarz et al., 2016
Reproduction	Arlian and Morgan, 2015
Feeding	Naegele et al., 2013
Anatomy	Wang et al., 2013; Yue-Ming et al., 2013; Wang et al., 2014
Molecular identification	Thet-Em et al., 2012
Genome analysis	Chan et al., 2015
Transcriptome	Peng et al., 2018
RNA interference	Yang et al., 2019
Microbiome	Lee et al., 2019
Human skin and clothes	Teplitsky et al., 2004
Humidity dependence	Arlian et al., 1999
Dispersal	Mollet and Robinson, 1996
Spatial distribution	van Asselt et al., 1996
Population structure	Dusbábek, 1979

Table 6.1 – Key features not related to medical, immunological effects, or (bio)control of the dust mite, *D. farinae*.

The epidemiology of the mites' allergy helps in exploration of the interaction between the organisms and humans at the population, individual, or molecular level. The prevalence data for allergy sensitisation vary from 65 to 130 million

individuals and among approximately fifty percent of asthmatic patients. Exposure to allergens may cause health complications due to the interrelationship between the immunity of a patient and the ecological strata. Inhaled mite aeroallergens activate the adaptive and innate immune responses, which offers novel mechanisms for intervention.

The genetic effects and genome of can be understood through recognition of the gene regulation and expression mechanisms to cause diseases and allergic reactions. RNA interference (RNAi) plays an important role in the regulation of gene expression in many organisms. The small RNA fragments can be classified as microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs), and short interfering RNAs (siRNAs). The miRNAs regulate functional genes affecting the developmental processes of an organism while siRNAs and piRNAs regulate non-functional transcripts, thus acting as a defence mechanism against transposable genetic elements.

Plants use siRNAs to silence the effects of transposable genetic elements. However, animals utilize piRNAs and siRNAs to silence the activity of the transposons. For instance, piRNAs have a crucial role in silencing transposons in vertebrates, insects, and flies. Evidently, most animals use secondary siRNAs made of RNA-dependent RNA polymerases to silence transposons. The authors believe that piRNAs in animals are usually involved in the silencing of the activities of transposons. Conversely, the house dust mites lack piRNA pathway, which is an essential mechanism for maintaining genome stability. The organisms utilise Dicer-dependent siRNAs to silence the transposons. The technique is currently applied for silencing transposons in animal systems.

Dicer leads to the formation of the miRNAs and siRNAs using double-stranded RNAs. The Dicer cleaves dsRNAs in house dust mites to generate 2-nt 3′ overhangs. The primary siRNAs form a 2-nt overlap between the antisense and 3′ ends of sense siRNAs. piRNAs play a crucial role in silencing the transposons in male germlines. The piRNAs are processed by the endonuclease zucchini from siRNAs. Nonetheless, the secondary piRNAs are generated from the targets through

a conserved ping-pong mechanism that utilises primary piRNA complex to cut the targets.

The piRNAs plays an important role in the shaping of the silencing mechanism of the transposons in males. The RNA may affect or lead to the silencing of transposable element directly or indirectly. In addition, it is important to note that piRNAs in many insects are directly involved in silencing the transposons. According to Li and Gu (2018), the application of high-throughput sequencing strategy lead to the construction of a complete genome for the house dust mite. Evidently, bioinformatics helps in the identification of Dicer alleles and Argonautes in males, which include DfaDcr1-3 and DfaAgo1-8, respectively. Nevertheless, the Argonauts bind to the piRNAs but do not belong to the clade of Piwi. As such, there is no piRNA pathway in the organisms.

D. farinae is an American house dust mite that belongs to the family Pyroglyphidae. The family has free-living and parasitic lineages. The organism is closely associated with vertebrates and it uses the most powerful enzymes to digest organic debris left by the vertebrates. For genomic sequencing, the microorganisms are cultured for 28 days on a maximal medium at a relative humidity of 75 % and room temperature. The organism is collected and separated from culture medium through sieving and separation through the saturated salt solution. The mites are then washed and subjected to starvation for one day before sterilization using 70 % concentrated alcohol. Prior to DNA extraction, the mites are washed and frozen in liquid nitrogen using a Promega genomic DNA purification kit. Lastly, the quality of extracted DNA is measured using, for example, Qubit dsDNA BR assay kit and its integrity analysed using agarose gel electrophoresis.

The genome size for *D. farinae* is determined through several stages that involve the preparation of sequencing libraries in which the collected data is trimmed for adapter and base call quality with Trimmomatic. Scaffolds are also generated through SSPACE and gaps closed through the application of a GapFiller. Evidently, the final assembly results in approximately 1,000 scaffolds with a GC content of 30.93 %. As such, the genome size of house dust mites was determined to be 70.76 Mb with an assembly gap of 3.14 %.

The gene prediction employs AUGUSTUS version 3.1.0, which is trained through a gene set of the organism. Gene functions are usually annotated with Pfam domains that utilise InterproScan version 5.3-46.0. The CEGMA version 2.5 can also be applied to identify the presence of the most crucial eukaryotic protein-coding genes. In addition, the secreted proteins are usually predicted using SignalP version 4.1 in which the transmembrane helices are determined using TMHMM server version 2.0. The gene prediction mechanism enables recognition of full-length sequences for most allergy-linked mites. This technique provides an easier mechanism of predicting the genes and their loci within the chromosome.

The allergy causing mechanism in *D. farinae* is due to the presence of proteins with a historical pattern of allergenicity. The serodominant specificities of the collective IgE binding to group 1&2 allergens are used to measure unequivocal sensitisation of the organisms than their extracts. However, discrepancies have been recognised in areas with complex acarofauna, thus creating the need for investigation of the specificity of the allergen compounds. Several groups of allergens inducing responses in approximately 40% of subjects are the mid-tier allergens that cover for the reminder IgE binding. It is believed that most allergens have different antibody binding mechanism in which the proteins in the body have a higher prevalence of binding in patients with atopic dermatitis.

Some allergens have been recognised as minor sensitisers through comparative quantitative assessment while others such as large lipid building proteins require further investigation. It is evident that the *D. farinae* gene sequence is used for the verification of paralogous and allelic variations. Identification and characterisation of *D. farinae* have been emphasised to enable for the development of appropriate and medically important allergen extracts. A deeper understanding and research on the regional amino acid sequence polymorphism is required for full characterisation of the organism as well as identification of essential and medically important products.

The identification of allergen gene sequence polymorphism in *D. farinae* was done using direct PCR and novel species-specific primers. Introns identified on the organism include ntpos 87 and 291 as well as the absence of intron 3 in house dust

mites. Detectable mutations in the American dust mites include thirteen silent and a novel non-synonymous mutation from Tryptophan W197 to Arginine R197. Approximately 30 proteins and macromolecules produce IgE-binding reactions in patients with allergic reactions caused by the mites. The organisms produce allergens known as cysteine proteases that induce the pro-inflammatory response through the breakage of lung epithelium.

Overall allergic reactions are dominated by the members of the family Dermatophagoides, which include *D. farinae* and *D. pteronyssinus*. Two species are closely related since they have approximately 80 % amino acid sequence identity. Nevertheless, the organisms produce different monoclonal antibodies, which are attributed to the position of IgE binding epitopes in the allergic molecules. The 4C1 anti Der f mAb binds to a conserved epitope to generate a set of amino acids. The organism usually produces amino acids such as glutamic acid, arginine, threonine, aspartic acid, and tyrosine among others. Evidently, the analysis of the amino acids assists in the prediction of cross-reactivity in allergens from *D. farinae*.

The genus *Dermatophagoides* belongs to the family of the Pyroglyphidae, which is listed under the Astigmata. The pyroglyphid mites inhabit animal nests. In the human environment, they are mainly located in the upholstery, textile floor covers and beddings, in which the primarily feed on the skin scale fraction in house dust. Dermatophagoides were recognised as a major source of allergens in house dust approximately forty years ago. The allergenic proteins present in mite are found in high concentrations, which become airborne after drying and pulverizing. In addition, the presence of allergens causes severe illnesses such as rhinitis, asthma, and dermatitis. In countries with a temperate climate, approximately a quarter of the population is sensitive to the effects and allergens derived from the house dust mites.

Evolutionary and population studies are facilitated by factors such as the complete mitochondrial genome sequences. The genome sequences are more informative and provide sets of genome-level characters such as mechanisms for controlling replication and transcription, gene loci, and RNA secondary structures. Conversely, the applicability of mitochondrial genomes as markers of highly

divergent lineages has not been understood to date. In addition, unravelling mt genomes are of the paramount essence since several chemical classes of insecticides and pesticides target the mitochondrial proteins. Distinguishable acaricides such as fluacrypyrim and acequinocyl affect mt electron transport by facilitating the inhibition of mitochondria-encoded cytochrome b in complex III. On that note, the economically important class of Mitochondrial Electron Transfer Inhibitors (METI)-acaricides usually focuses on the mitochondrial complex I, although their exact molecular target is yet to be understood. Nevertheless, resistance to the acaricide bifenazate is caused by mutations in the mitochondria encoded cytochrome b and evolved through a short stage of mt heteroplasmy rapidly. Currently, the mitochondrial genomes of 20 species belonging to the Acari have been identified and characterised. Most of the submitted sequences have features typical to the metazoan mitochondrial genomes. The sequences are circular and contain a coding region with 37 genes between 13 and 20 kb in length and a small non-coding region. The coding region is mostly AT-rich that plays a crucial role in the initiation of transcription and replication.

The transfer ribonucleic acid (tRNA) is an RNA that decodes a messenger RNA (mRNA) sequence into a protein. The tRNAs function at specific sites in the ribosome during translation. The translation entails the synthesis of a protein from a mRNA molecule. In *D. farinae* only one tRNA lacks the D-arm: trnS1, which is a common feature in most metazoans (Dermauw et al., 2009). With the exception of trnC, trnV and trnS1, all tRNAs have T-arm variable replacement loops instead of the T-arm, which are also found in the tRNAs of other organisms such as the L. pallidum and S. magnus. The absence of the T-arm is a common feature for tRNAs of Araneae, Thelyphonida, and Scorpiones. The changes in mitochondrial ribosomes due to the loss of arms from tRNAs starts shortly after the occurrence of changes in specific elongation factors.

The mitochondrial genome of *D. farinae* is a circular DNA molecule that contains more than 14,000 bp. The mitochondrial genome of *D. pteronyssinus*, which is similar to that of *D. farinae*, contains a complete set of 37 genes. The genes are segmented to 13 protein-coding genes, 2 rRNA genes, and 22 tRNA genes, which are

commonly found in metazoan mitochondrial genomes. The mitochondrial gene order has numerous differences compared to other Acari mitochondrial genomes. In comparison with the mitochondrial genome of *Limulus polyphemus*, which is usually considered as the ancestral arthropod pattern, only a few gene boundaries are conserved. The majority strand has more than 70 % of AT-content but a GC-skew of 0.194. The skew is a reverse of the normally recognised for the typical animal mitochondrial genome. A microsatellite is usually present in the large non-coding region containing 286 bp, which acts as the control region. Most tRNA genes do not have a T-arm, which provokes the formation of canonical cloverleaf tRNA-structures with a reduced size.

The mitochondrial genome of *D. farinae* shares different features with previously characterised *D. pteronyssinus* mitochondrial genomes. The gene order is rearranged and represents a new pattern within the Acari. In this case, the tRNAs and rRNAs are reduced, which corroborates functional co-evolution theory of the two molecules. Additionally, the strong and reversed GC- and AT-skews of the molecules and the organism shows an inversion of the control region as an evolutionary event. This analysis offers a clear description of the complete mt genome of a member of the Astigmata, which is listed under the Sarcoptiformes. However, the length, gene, and AT-content of the organism of *D. farinae* and *D. pteronyssinus* are similar to mtDNA of other members of the Acari, thus, the mt genome of the organisms has interesting traits. The gene order of *D. pteronyssinus* is completely different from that of other Acari mitochondrial genomes. In addition, the rRNAs of *D. farinae* is shorter and all transfer RNAs lacks the T-arm in comparison with other parasitiform mites.

Several methods such as physical, chemical, and biological are used to prevent the spread of house dust mites. The diverse nature of house dust mites has rendered these methods ineffective to control them. House dust mites can be regulated and controlled by replacing the natural fibre beddings with the synthetic ones. This interferes with the mites' ecological niches forcing them to migrate or in extreme cases, die due to lack of shelter and food. Secondly, the mites can be controlled by utilising the scouting and trapping methods. This involves the

physical identification of the mites with the help of hand lens and magnifying lens to detect the location of the mites in the house. Thirdly, chemical measures such as miticidal, insecticidal, dominant, and horticultural oils significantly control the population of house dust mites in an ecosystem. Dormant oils are mainly used to kill the mites' eggs, larva, and pharate adults before they fully mature. For instance, Nuke Em, an organic insecticide kills eggs and larval stages through dehydrating and interfering with the body metabolism. However, this compound leaves a trace on the beddings and its advisable to soak the beddings in hot water before spraying the house. Lastly, covering and vacuuming of mattresses regularly helps to kill the house dust mites that inhabit these places. This lowers the quantity of sweat in the beddings and thus reducing the amount of food for the mites. As a result, either the mites are forced to migrate to another habitat, or they die off due to starvation.

6.2 Materials and Methods

The genome of *D. farina* has been comprehensively analyzed for microsatellites using the Geneious platform, version R10, from Biomatters. Microsatellites were identified in the genome with Phobos Tandem Repeat Finder for Geneious, version 1.0.6, by Christoph Mayer. Primers were designed with the help of Primer 3.

To aid the visualization of the PCR product, a universal tag should be added to the 5'-end of the forward primer. When doing so, bases that are common between the 3'-end of the tag and 5'-end of the primer sequence should not be duplicated. A total of ten tags have been analysed for this mite genome. The two tags with the least correspondence in the target genome are M13 mod B, 5'- CAC TGC TTA GAG CGA TGC -3', and Tail A, 5'- GCC TCC CTC GCG CCA -3' and the most commonly used tags are CAG, 5'- CAG TCG GGC GTC ATC A -3', and M13R, 5'- GGA AAC AGC TAT GAC CAT -3'. The tags range in length between 15 and 18 nt, this should be taken into account while choosing the annealing temperature during the second part of the PCR reaction. The reverse primers should be amended at the 5'-end with a partial of half PIG tail: 5'- GTT T -3'. For the first five single-primer primer pairs, all four tags have been added, for all subsequent primers, only the M13 mod B tag and the partial PIG tail have been added.

Please note that the fluorophore primer, which acts as forward primer during the second part of the PCR and the universal tag of the specific forward primer must be identical, not their reverse compliment, i.e.

Fluorophore primer:

fluorophore – cactgcttagagcgatgc -3'

Tagged forward primer for Df MS1 F:

cactgcttagagcgatgcACAACCCAATTCAAATGGATCCA

M13B F	18 + 3 = ~ 21
TailA F	14 + 3 = ~ 17
CAG F	15 + 3 = ~ 18
M13R	18 + 3 = ~ 21

For choosing the most appropriate tag, please see Table. 6.2

Analysis of tags searched against whole-genome shotgun contigs

CAG	16 nt	13 nucleotides identical	2 matches	26.3 bits
		12	5	24.3
		11	43	22.3
		10		20.3
M13R	18	14	1	
		13	2	
		12	10	
		11	75	
M13(-21)	18	14	2	
		13	3	
		12	26	
		11	>75	
M13 mod A	18	12	9	
		11	28	
M13 mod B	18	12	1	24.3
		11	10	22.3
T7term	19	14	1	
		13	2	
		12	13	
		11	68	
Tail A	15	11	1	
		10	11	
Tail B	15	12	1	
		11	3	
		10	29	
Tail C	18	11	15	
		10	60	
Tail D	17	12	2	24.3
		11	5	22.3
		10	10	20.3

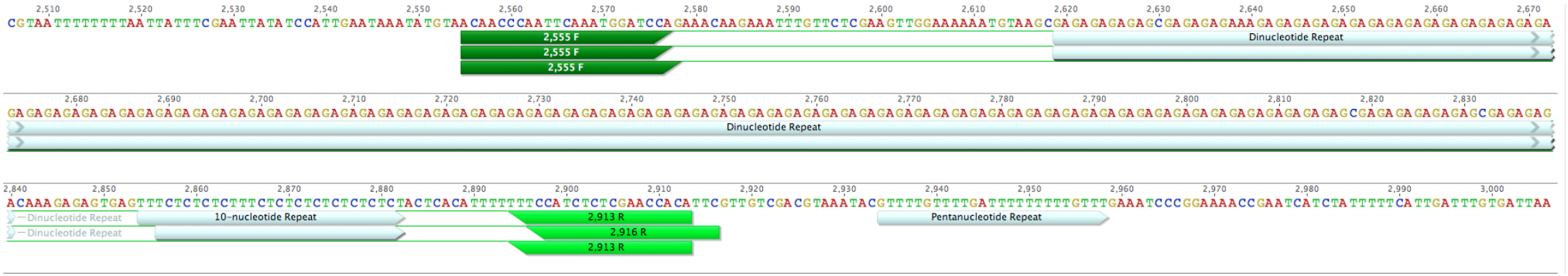
Table 6.2 – Distribution of individual stags in the genome of *D. farinae*. The more matches with the highest number of nucleotides a particular tag to the genome of the species in which it will be used has, the worse it is likely to perform.

6.3 Results

Table 6.2 lists all microsatellites in detail developed with all pertinent characteristics for *D. farinae*. Specific primer pairs including tags and tails are listed. The assumed quality of the microsatellites has been scored with Phobos value. For each microsatellite, two alternative primer pairs are depicted in the alignment of the microsatellite to the genome fragment as well. The microsatellites used to investigate the most appropriate tag for *D. farinae* show the sequences of all tags, the other microsatellites only include sequences with M13 B tag.

Proposed microsatellites for *Dermatophagoides farinae*

Dinucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
1	ASGP 01 010 258	AG	GA	111	222	2,619-2,840	196	98	F: ACAACCCAATTCAAATGGATCCA R: TGTGGTTCGAGAGATGGAAA	58.8 56.5	359
Df MS 1 M13B F cactgcttagagcgatgcACAACCCAATTCAAATGGATCCA Df MS 1 TailA F gcctccctcgcgccACAACCCAATTCAAATGGATCCA Df MS 1 CAG F cagtcgggcgtcatcACAACCCAATTCAAATGGATCCA Df MS 1 M13R F ggaaacagctatgaccatACAACCCAATTCAAATGGATCCA Df MS 1 R gttTGTGGTTCGAGAGATGGAAA											



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
2	ASGP 01 002 253	AG	TC	126	153	5,057-5,208	133	98	F: TCCGCCTCATCATCATCAACA R: TCCCTGTTGTACCGTCAAGT	59.5 58.5	347

Df MS 2 M13B F

cactgcttagagcgatgcTCCGCCTCATCATCATCAACA

Df MS 2 TailA F

gcctccctcgcgccaTCCGCCTCATCATCATCAACA

Df MS 2 CAF F

cagtcgggcgtcatcaTCCGCCTCATCATCATCAACA

Df MS 2 M13R F

ggaaacagctatgaccaTCCGCCTCATCATCATCAACA

Df MS 2 R

gttTCCCTGTTGTACCGTCAAGT

Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
3	ASGP 01 000 162	AG	TG	73	147	3,500-3,645	133	98	F1: ACCGTTCCATAGTCCTCCCA F2: TTCAAGCCCGGTACACACAA R: TTGCGCACGATTCTTTCTGC	60.0 59.8 60.1	 500 438

Two different combinations of primers.

Df MS 3 M13B F1

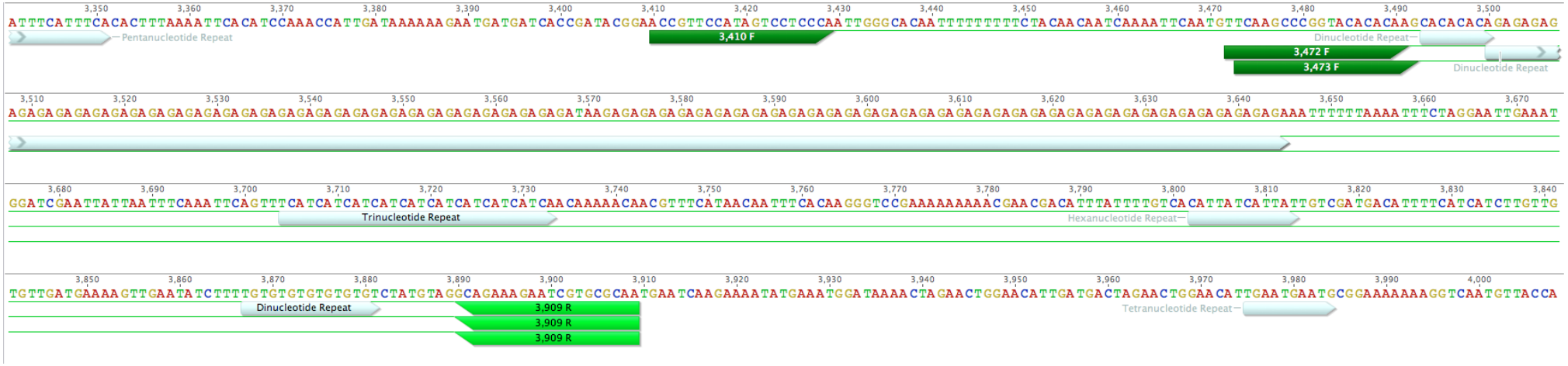
cactgcttagagcgatgcACCGTTCCATAGTCCTCCCA

Df MS 3 M13B F2

cactgcttagagcgatgcTTCAAGCCCGGTACACACAA

Df MS 3 R

gtTTGCGCACGATTCTTTCTGC



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
5	ASGP 01 002 633	AG	AG	67	135	2,008-2,141	127	99	F1: TGTCTTCAAGTTTCATAGATTTCCA R1: TCCACTTGCTTTGGGCTTCT F1: TGTCTTCAAGTTTCATAGATTTCCA R2: TTGGGGGCAATATGAAGGGG F2: TCATAGATTTCCAATGAATCACC R2: TTGGGGGCAATATGAAGGGG	56.7 59.8 59.7 54.6	347 415 366

Three different combinations of primers.

Df MS 5 M13B F1

cactgcttagagcgatgcTGTCTTCAAGTTTCATAGATTTCCA

Df MS 5 M13B F2

cactgcttagagcgatgcTCATAGATTTCCAATGAATCACC

Df MS 5 R1

gttTCCACTTGCTTTGGGCTTCT

Df MS 5 R2

gtTTGGGGGCAATATGAAGGGG

Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
6	ASGP 01 004 004	AG	TG	65	131	6,032-6,163	100	96	F: TGGCCAAGCTAAACAACACA R1: TCACGACAACAGTATGATCGA R2: TGCTATTGAATCACGACAACAGT	58.2 56.2 58.7	 318 328

Two different combinations of primers.

Df MS 6 M13B F

cactgcttagagcgatgcTGGCCAAGCTAAACAACACA

Df MS 6 R1

gttTCACGACAACAGTATGATCGA

Df MS 6 R2

gttTGCTATTGAATCACGACAACAGT

Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
7	ASGP 01 006 416	AG	CT	65	130	1,316-1,445	110	98	F: CGGATTCTGCGTCCTGTGTA R: TTGGTGGCAGCAACAACAAC	59.8 60.1	400

Df MS 7 M13B F

cactgcttagagcgatgCGGATTCTGCGTCCTGTGTA

Df MS 7 TailA F

gcctccctcgcgccaCGGATTCTGCGTCCTGTGTA

Df MS 7 CAF F

cagtcgggcgtcatcaCGGATTCTGCGTCCTGTGTA

Df MS 7 M13R F

ggaaacagctatgaccatCGGATTCTGCGTCCTGTGTA

Df MS 7 R

gtTTGGTGGCAGCAACAACAAC

Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
9	ASGP 01 001 583	AC	CA	61	124	3,661-3,783	110	98	F: AAAAACAACAGCCACCGTGG R1: GTTGACGATCCAGAACGGGA R2: GCGAATGCGTAAATCGTCGT	59.8 59.8 59.7	 386 320

Two different combinations of primers.

Df MS 9 M13B F

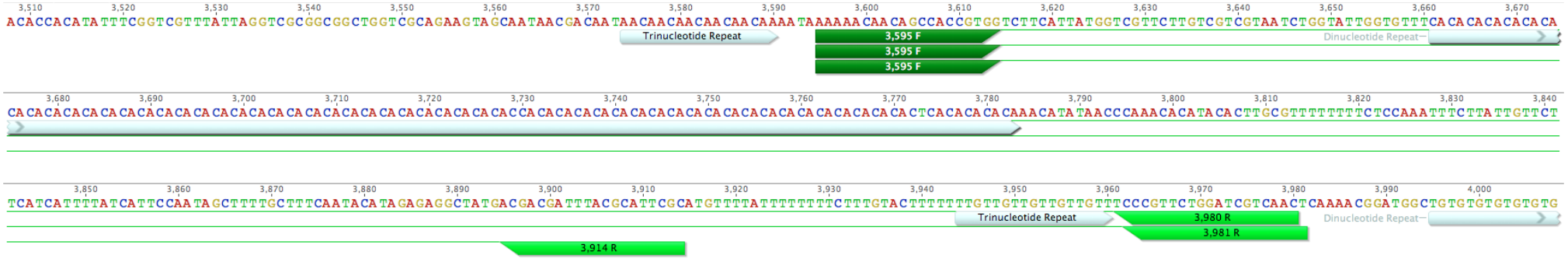
cactgcttagagcgatgcAAAAACAACAGCCACCGTGG

Df MS 9 R1

gtttGTTGACGATCCAGAACGGGA

Df MS 9 R2

gtttGTTGACGATCCAGAACGGGA



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
10	ASGP 01 003 948	AG	TC	60	121	1,209-1329	119	100	F1: AGGTTGCCGACATCTGTTCA F2: GTGTGTGCCGCGGAAAATAT F3: TAGTATCACGTGTGTGCCGC R: AAAATCCTAACCGCACGCAC	59.6 59.6 60.5 59.5	 249 334 343

Three different combinations of primers.

Df MS 10 M13B F1

cactgcttagagcgatgcAGGTTGCCGACATCTGTTCA

Df MS 10 M13B F2

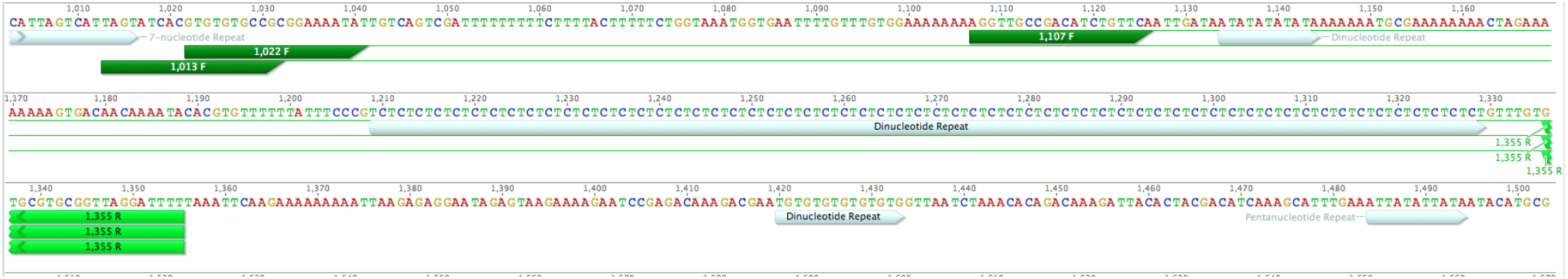
cactgcttagagcgatgcGTGTGTGCCGCGGAAAATAT

Df MS 10 M13B F3

cactgcttagagcgatgcTAGTATCACGTGTGTGCCGC

Df MS 10 R

gtttAAAATCCTAACCGCACGCAC



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
11	ASGP 01 001 586	AC	CA	60	122	962-1,082	114	99	F: ACACGCAGCATCTCAAATGAC R: TGACATTCATACACACGCACA	59.5 58.2	389

Df MS 11 M13B F

cactgcttagagcgatgcACACGCAGCATCTCAAATGAC

Df MS 11 TailA F

gcctccctcgcgccACACGCAGCATCTCAAATGAC

Df MS 11 CAG F

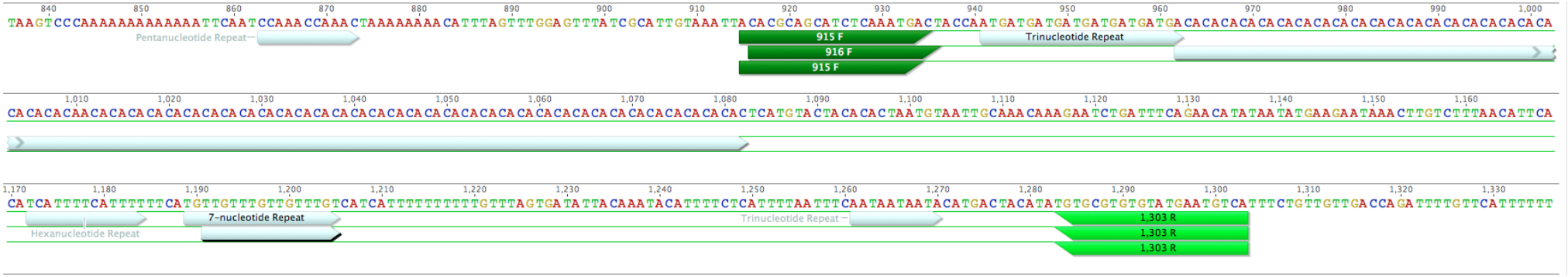
cagtcgggcgtcatcACACGCAGCATCTCAAATGAC

Df MS 11 M13R F

ggaaacagctatgaccatACACGCAGCATCTCAAATGAC

Df MS 11 R

gttTGACATTCATACACACGCACA



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
12	ASGP 01 010 696	AG	AG	57	115	388-501	83	96	F: TCGTCAATACACCGACTCGA R: AGAGAGTGGGCGCAAAAGAA	58.6 59.9	254

Df MS 12 M13B F
cactgcttagagcgatgcTCGTCAATACACCGACTCGA
Df MS 12 TailA F
gcctccctcgcgccaTCGTCAATACACCGACTCGA
Df MS 12 CAG F
cagtcgggcgtcatcaTCGTCAATACACCGACTCGA
Df MS 12 M13R F
ggaaacagctatgaccaTCGTCAATACACCGACTCGA
Df MS 12 R
gtttAGAGAGTGGGCGCAAAAGAA

Dinucleotide repeats

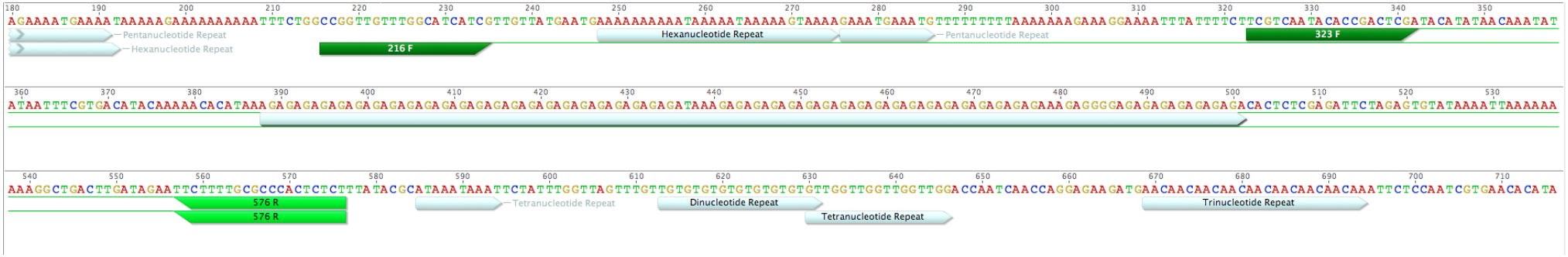
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
13	ASGP 01 002 874	AG	AG	57	114	388-501	83	96	F: CCGGTTGTTTGGCATCATCG same as in MS12	60.2 59.9	361

Df MS 13 M13B F

cactgcttagagcgatgCCGGTTGTTTGGCATCATCG

Df MS 12 R

gtttAGAGAGTGGGCGCAAAAGAA



Dinucleotide repeats

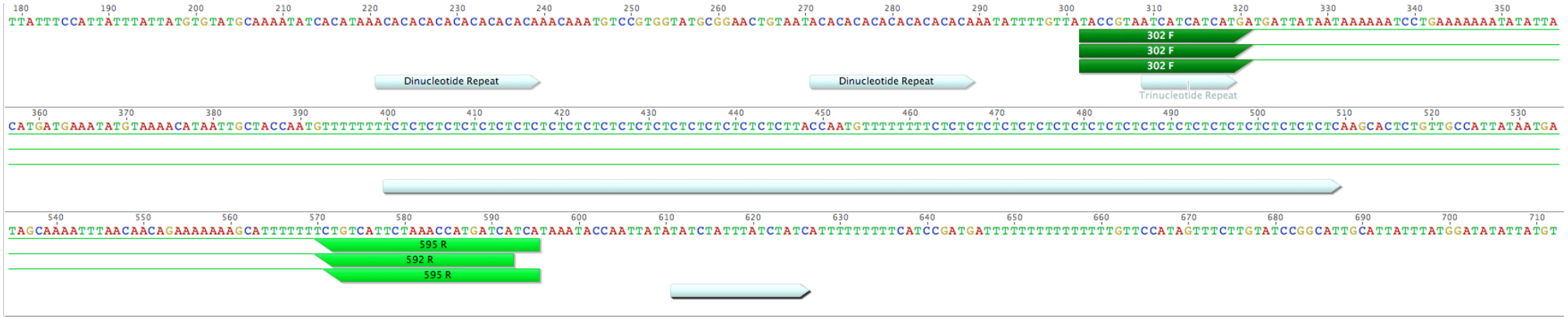
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
14	ASGP 01 003 649	AG	TC	55	112	400-509	51	91	F: TACCGTAATCATCATCATGA R: TGATGATCATGGTTTAGAATGACAGA	51.2 58.4	291

Df MS 14 M13B F

cactgcttagagcgatgcTACCGTAATCATCATCATGA

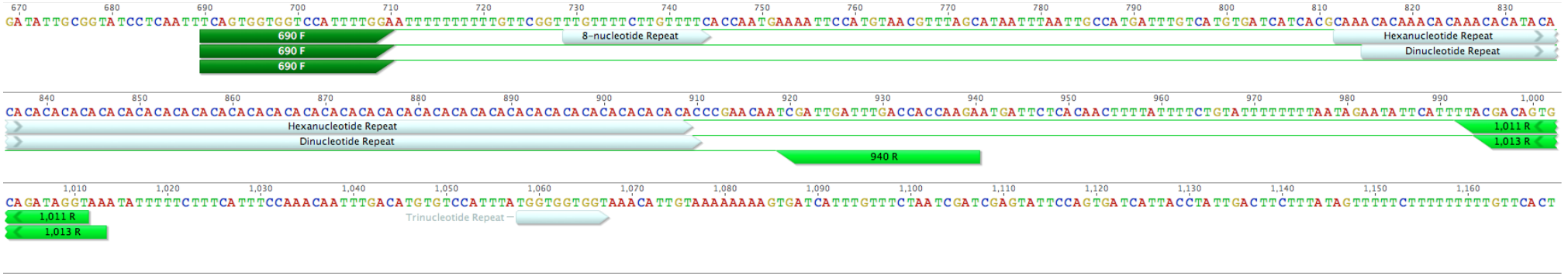
Df MS 14 R

gttTGATGATCATGGTTTAGAATGACAGA



Dinucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
18	ASGP 01 000 930	AG	AG	33	97	5,122-5,220	97	100	F: GTGTGCGCGCGATTATTTGA R: ACGTAAATCAAGCTCCGCCT	60.2 59.8	193
Df MS 18 M13B F cactgcttagagcgatgcGTGTGCGCGCGATTATTTGA Df MS 18 R gtttACGTAAATCAAGCTCCGCCT											

Dinucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
19	ASGP 01 008 617	AC	AC	48	96	812-909	76	97	F: TCAGTGGTGGTCCATTTTGGA R1: ACCTATCTGCACTGTCGTAA R2: TCTTGGTGGTCAAATCAATCGA	59.5 60.0 58.0	 322 251
Two different combinations of primers.											
Df MS 19 M13B F cactgcttagagcgatgcTCAGTGGTGGTCCATTTTGGA Df MS 19 R1 gtttACCTATCTGCACTGTCGTAA Df MS 19 R2 gttTCTTGGTGGTCAAATCAATCGA											



The hexanucleotide repeat has 86.7 % perfection, while the dinucleotide repeat has 96.9 % perfection, therefore it is treated as a dinucleotide repeat.

Dinucleotide repeats

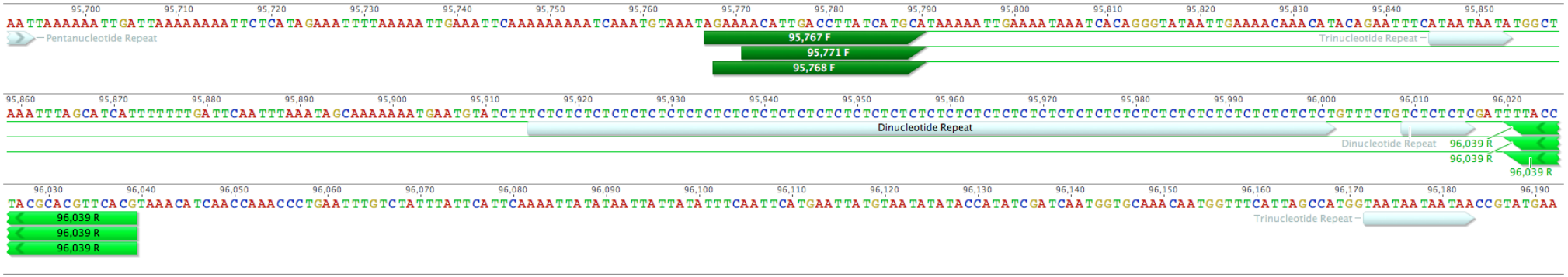
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
32	ASGP 01 003 642	AG	TC	43	87	95,915-96,001	85	100	F: AGAAAACATTGACCTTATCATGCA R: CGTGAACGTGCGTAGGTAAA	57.2 58.6	273

Df MS 32 M13B F

cactgcttagagcgatgcAGAAAACATTGACCTTATCATGCA

Df MS 32 R

gtttCGTGAACGTGCGTAGGTAAA



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
34	ASGP 01 008 377	AG	AG	42	85	8,398-8,483	48	93	F: GATGTTGTTTCCACACCGCC R1: AAAAACGACAATGTCCGGCC R2: TCCGGCCGTTAGTGGAAAAT R3: GACAATGTCCGGCCGTTAGT	60.0 59.7 59.7 60.4	 279 266 273

Three different combinations of primers.

Df MS 34 M13B F

cactgcttagagcgatgcGATGTTGTTTCCACACCGCC

Df MS 34 R1

gtttAAAAACGACAATGTCCGGCC

Df MS 34 R2

gttTCCGGCCGTTAGTGGAAAAT

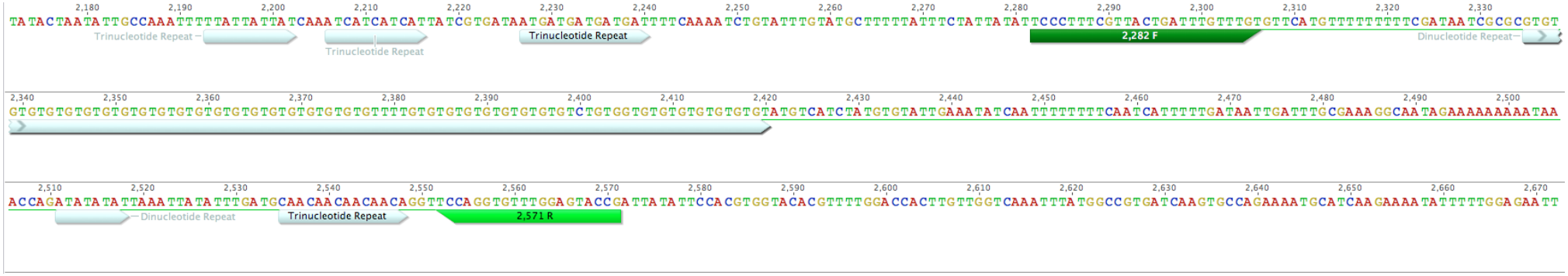
Df MS 34 R3

gtttGACAATGTCCGGCCGTTAGT

Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
35	ASGP 01 002 102	AC	GT	44	88	2,335-2,420	62	95	F1: TCCCTTTCGTTACTGATTTGTTTGT R: CGGTACTCCAAACACCTGGA	59.4 59.3	290

Df MS 35 M13B F
cactgcttagagcgatgcTCCCTTTCGTTACTGATTTGTTTGT
Df MS 35 R
gtttCGGTACTCCAAACACCTGGA



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
39	ASGP 01 006 423	AC	CA	42	84	11,472-11,555	82	100	F1: TGCACGTGCTAATAGCCTTGA F2: TGTTGCACGTGCTAATAGCC R: GGTCAAATGCACACAGGTAGC	56.1 58.9 59.8	 270 273

Two different combinations of primers.

Df MS 39 M13B F1

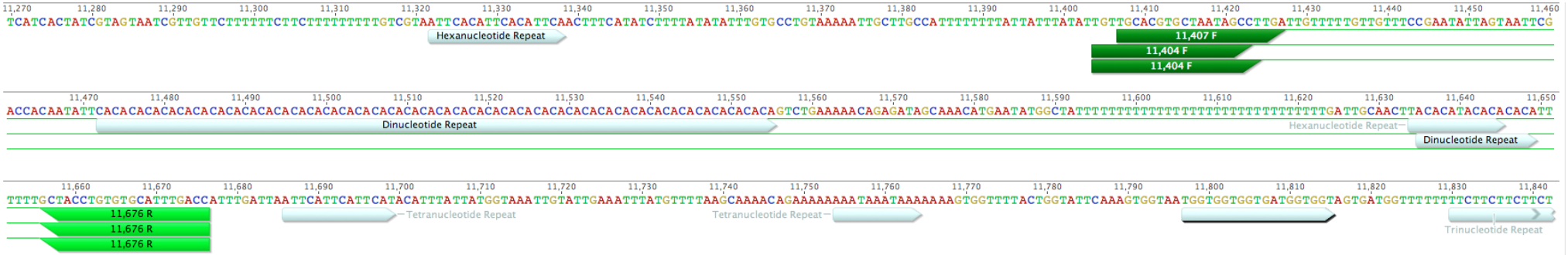
cactgcttagagcgaTGCACGTGCTAATAGCCTTGA

Df MS 39 M13B F2

cactgcttagagcgatgcTGTTGCACGTGCTAATAGCC

Df MS 39 R

gtttGGTCAAATGCACACAGGTAGC



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
41	ASGP 01 000 157	AC	CA	42	84	33,771-33,854	82	100	F1: GCCATGTATAATGATAATTCGTTTGC F2: TTCAATTCGATTCGTTGAAA R: CCATCATCGTCATCAACAAGTGG	57.8 51.7 59.9	 249 215

Two different combinations of primers.

Df MS 41 M13B F1

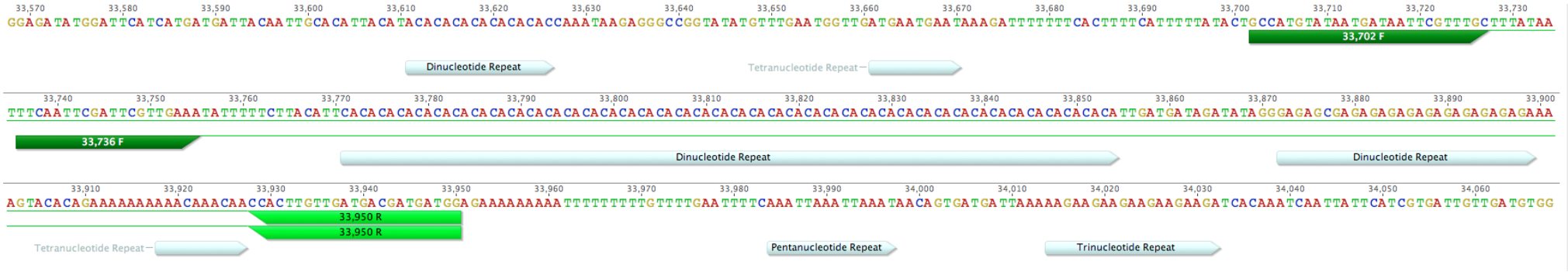
cactgcttagagcgatGCCATGTATAATGATAATTCGTTTGC

Df MS 41 M13B F2

cactgcttagagcgatgcTTCAATTCGATTCGTTGAAA

Df MS 41 R

gtttCCATCATCGTCATCAACAAGTGG



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
43	ASGP 01 009 489	AG	AG	41	83	35-117	81	100	F: CAGAAAAGAAGTGTGATTCCT R1: TGGATAATTCAACATCAGATGGA R2: AGATGGATAATTCAACATCAGATGGA	53.7 55.4 58.2	 311 314

Two different combinations of primers.

Df MS 43 M13B F

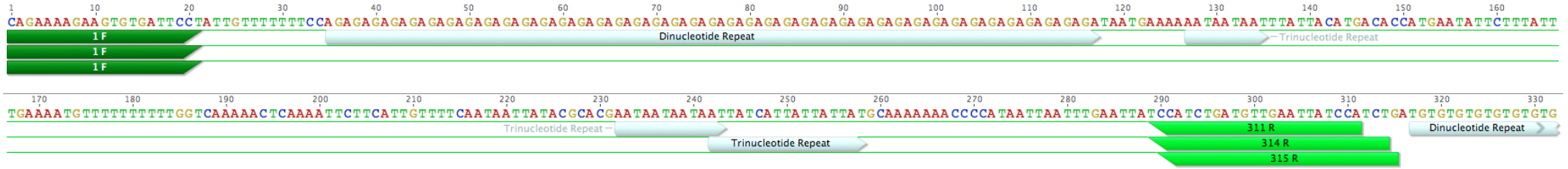
cactgcttagagcgatgCAGAAAAGAAGTGTGATTCCT

Df MS 43 R1

gttTGGATAATTCAACATCAGATGGA

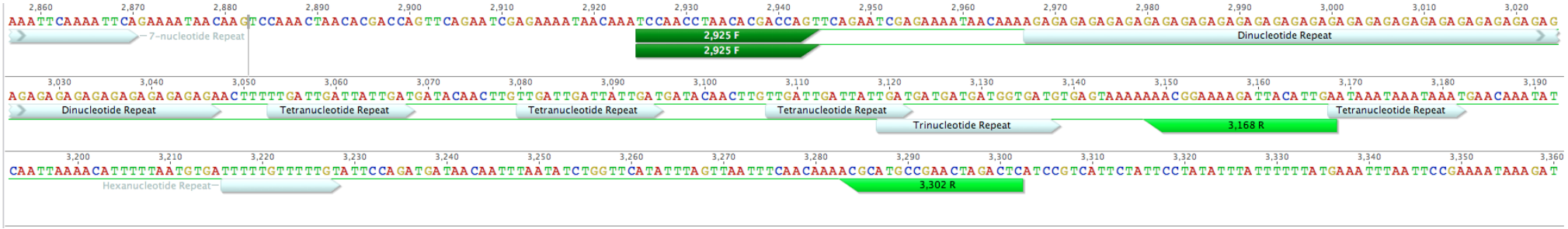
Df MS 43 R2

gtttAGATGGATAATTCAACATCAGATGGA



Dinucleotide repeats											
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm °C	Prod. bp
44	ASGP 01 001 448	AG	TC	41	83	2,757-2,839	81	100	F1: TGACCGACATTGACTTGTTTGAC F2: TGATGACCGACATTGACTTGT R: ACAAGATTTTGGATCAATCGCCA	59.7 59.7 59.2	 237 240
Two different combinations of primers.											
Df MS 44 M13B F1 cactgcttagagcgatgcTGACCGACATTGACTTGTTTGAC Df MS 44 M13B F2 cactgcttagagcgatgcTGATGACCGACATTGACTTGT Df MS 44 R gtttACAAGATTTTGGATCAATCGCCA											

Dinucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
46	ASGP 01 011 446	AG	AG	40	81	2,967-3,047	79	100	F: TCCAACCTAACACGACCAGT R1: GAGTCTAGTTCGGCATGCGT R2: TCAATGTAATCTTTTCCGTTT	58.6 60.2 51.7	219 378 244
Two different combinations of primers.											
Df MS 46 M13B F cactgcttagagcgatgcTCCAACCTAACACGACCAGT Df MS 46 R1 gtttGAGTCTAGTTCGGCATGCGT Df MS 46 R2 gttTCAATGTAATCTTTTCCGTTT											



Dinucleotide repeats

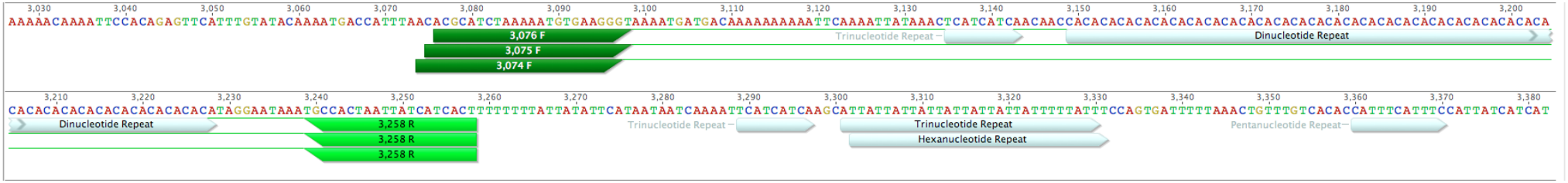
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
51	ASGP 01 002 364	AC	CA	40	80	3,149-3,228	78	100	F: ACGCATCTAAAAATGTGAAGGGT R: AGTGATGATAATTAGTGGCA	58.9 51.6	183

Df MS 51 M13B F

cactgcttagagcgatgcACGCATCTAAAAATGTGAAGGGT

Df MS 51 R

gtttAGTGATGATAATTAGTGGCA



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
77	ASGP 01 002 860	AG	TC	39	79	1,357-1,435	77	100	F: ACAAGTTGATGAGACATTACATCGT R1: CCTTGATGGCCGGAAAAGTG R2: TGATGGCCGGAAAAGTGAGA	58.8 59.7 59.3	 157 154

Two different combinations of primers.

Df MS 77 M13B F

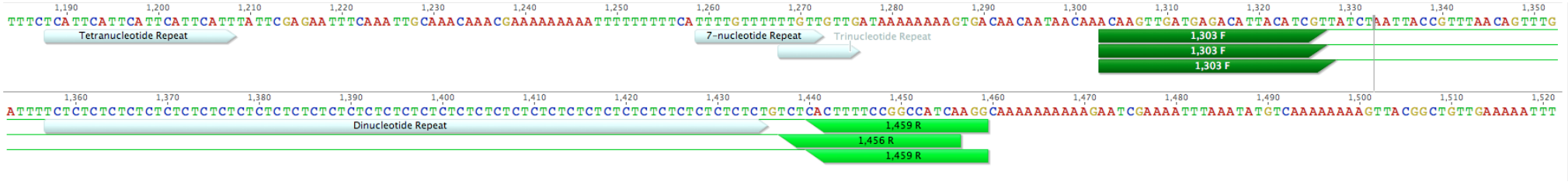
cactgcttagagcgatgcACAAGTTGATGAGACATTACATCGT

Df MS 77 R1

gtttCCTTGATGGCCGGAAAAGTG

Df MS 77 R2

gttTGATGGCCGGAAAAGTGAGA



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
79	ASGP 01 003 202	AG	AG	38	76	4,615-4,690	74	100	F1: TCATCATAATCAATCCGTTCAGCT F2: ACCCGAATCCATCTAAAGAATGA R: AATCACATGTGTGTCTGCGC	58.4 57.4 59.5	 126 190

Two different combinations of primers.

Df MS 79 M13B F1

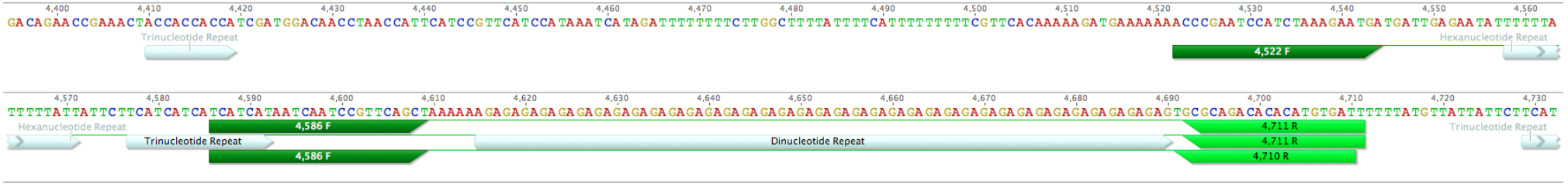
cactgcttagagcgatgcTCATCATAATCAATCCGTTCAGCT

Df MS 79 M13B F2

cactgcttagagcgatgcACCCGAATCCATCTAAAGAATGA

Df MS 79 R

gtttAATCACATGTGTGTCTGCGC



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
80	ASGP 01 003 457	AG	TC	37	75	6,245-6,319	73	100	F1: AACATCTGTTTTGCTTGACAAGC F2: AGCAGCAACAATTTTTCAGTGT R: ACTCACTGCCATAGTCGACG	58.9 57.9 59.5	 221 201

Two different combinations of primers.

Df MS 80 M13B F1

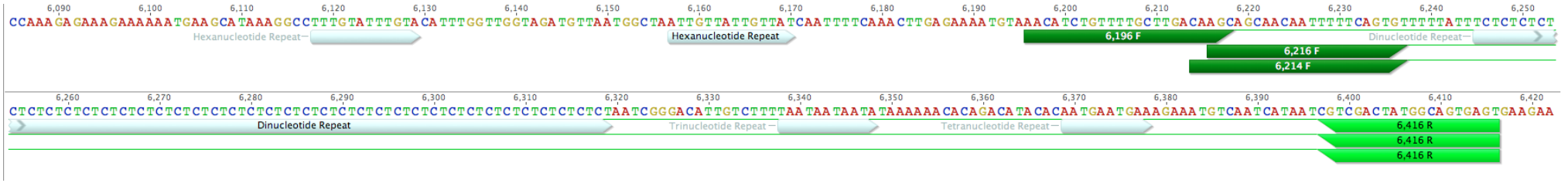
cactgcttagagcgatgcAACATCTGTTTTGCTTGACAAGC

Df MS 80 M13B F2

cactgcttagagcgatgcAGCAGCAACAATTTTTCAGTGT

Df MS 80 R

gtttACTCACTGCCATAGTCGACG



Dinucleotide repeats

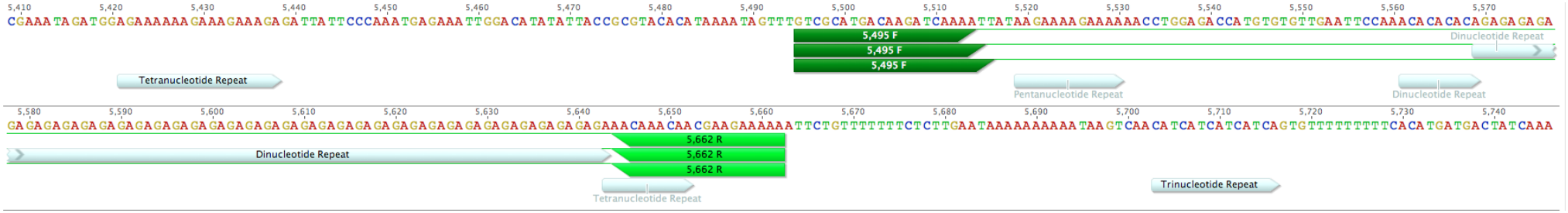
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
81	ASGP 01 002 196	AG	AG	37	75	5,569-5,643	73	100	F: TCGCATGACAAGATCAAAA R: TTTTTCTTCGTTGTTTGTT	54.6 49.4	168

Df MS 81 M13B F

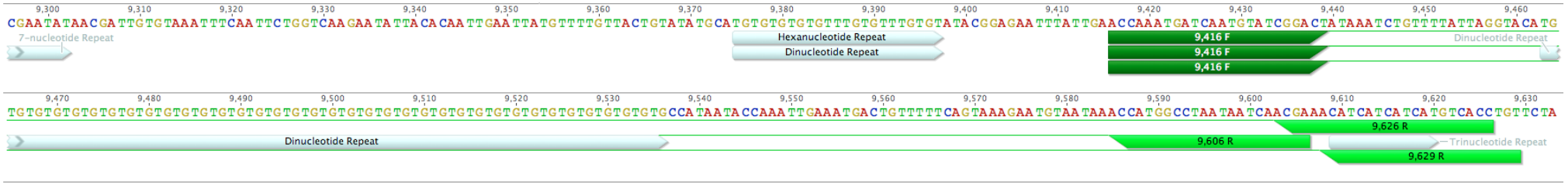
cactgcttagagcgatgcTCGCATGACAAGATCAAAA

Df MS 81 R

gTTTTTCTTCGTTGTTTGTT



Dinucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
82	ASGP 01 005 208	AC	TC	37	74	9,463-9,536	72	100	F: ACCAAATGATCAATGTATCGGACT R: TCGTTGATTATTAGGCCATGGT	58.3 57.6	191
Df MS 82 M13B F cactgcttagagcgatgcACCAAATGATCAATGTATCGGACT Df MS 82 R gttTCGTTGATTATTAGGCCATGGT											



Dinucleotide repeats

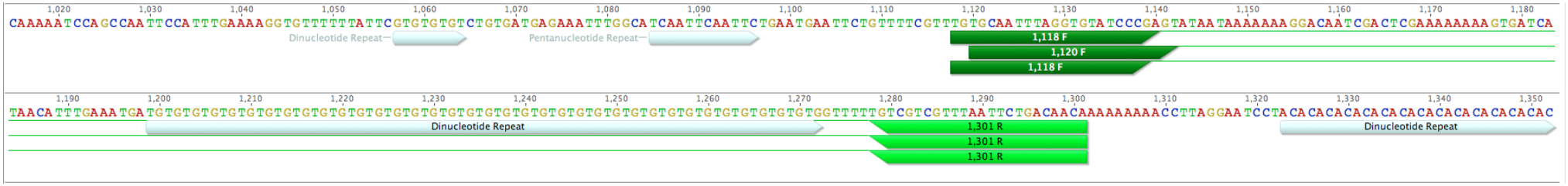
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
83	ASGP 01 003 296	AC	TG	37	74	1,199-1,272	72	100	F: TGTGCAATTTAGGTGTATCCCGA R: TGTTGTCAGAATTAAACGACGACA	60.1 59.2	184

Df MS 83 M13B F

cactgcttagagcgatgcTGTGCAATTTAGGTGTATCCCGA

Df MS 83 R

gttTGTTGTCAGAATTAAACGACGACA



Dinucleotide repeats

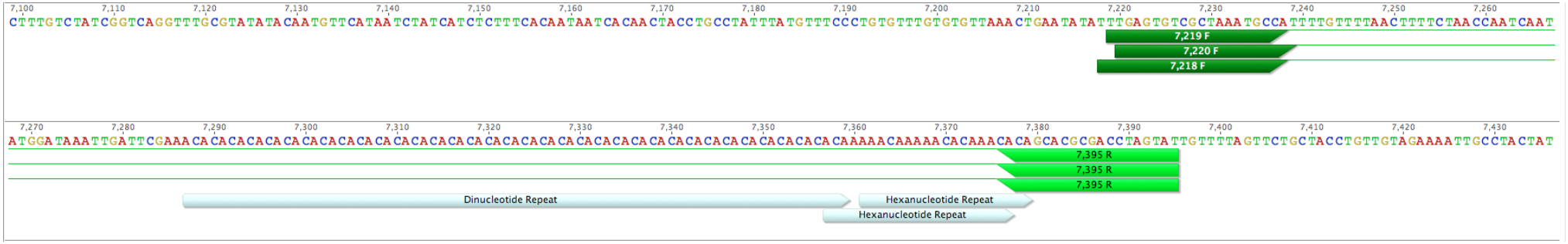
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
84	ASGP 01 004 048	AC	AC	36	73	7,287-7,359	71	100	F: TTGAGTGTCGCTAAATGCCA R: ATACTAGGTCGCGTGCTGTG	57.8 59.9	177

Df MS 84 M13B F

cactgcttagagcgatgcTTGAGTGTCGCTAAATGCCA

Df MS 84 R

gtttATACTAGGTCGCGTGCTGTG



Dinucleotide repeats

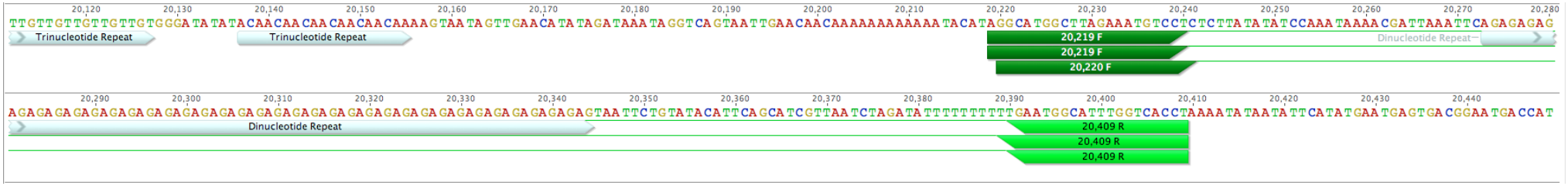
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
85	ASGP 01 006 174	AG	AG	36	72	20,273-20,344	70	100	F: AGGCATGGCTTAGAAATGTCCT R: AGGTGACCAAATGCCATTCA	59.8 57.7	191

Df MS 85 M13B F

cactgcttagagcgatgcAGGCATGGCTTAGAAATGTCCT

Df MS 85 R

gtttAGGTGACCAAATGCCATTCA



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
86	ASGP 01 007 346	AC	TC	34	69	2,335-2,403	70	100	F: GGCACCACAAGAAGAAATGATGA R: AACTCGAGCCTACAACCTACA	59.5 58.5	181

Df MS 86 M13B F
cactgcttagagcgatgcGGCACCACAAGAAGAAATGATGA
Df MS 86 R
gtttAACTCGAGCCTACAACCTACA

Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
87	ASGP 01 006 661	AG	AG	34	69	12,718-12,786	67	100	F1: TGACTCAATGAAAGACTAACCT R1: TCAAATTTCGTGCATCAAGAGT F1: TGACTCAATGAAAGACTAACCT R2: TGTCGATATCAAATTTCGTGCA F2: TCCAGATATGACTCAATGAAAGACT R2: TGTCGATATCAAATTTCGTGCA	54.8 56.9 56.8 57.6	133 141 141

Three different combinations of primers.

Df MS 87 M13B F1

cactgcttagagcgatgcTGACTCAATGAAAGACTAACCT

Df MS 87 M13B F2

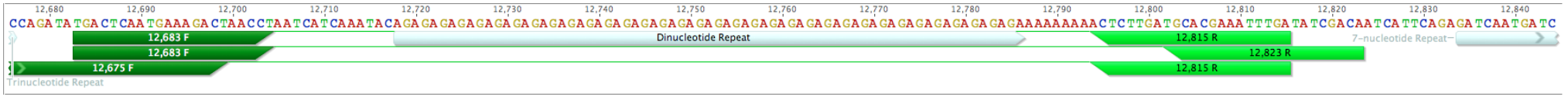
cactgcttagagcgatgcTCCAGATATGACTCAATGAAAGACT

Df MS 87 R1

gttTCAAATTTCGTGCATCAAGAGT

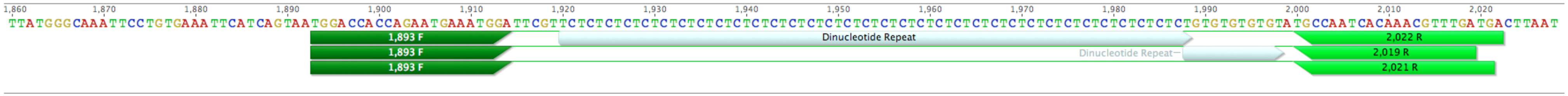
Df MS 87 R2

gttTGTCGATATCAAATTTCGTGCA



Dinucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
88	ASGP 01 000 183	AG	TC	34	69	1,920-1,988	67	100	F: TGGACCACCAGAATGAAATGGA R: TCATCAAACGTTTGTGATTGGCA	59.6 59.9	130

Df MS 88 M13B F
cactgcttagagcgatgcTGGACCACCAGAATGAAATGGA
Df MS 88 R
gttTCATCAAACGTTTGTGATTGGCA



Dinucleotide repeats

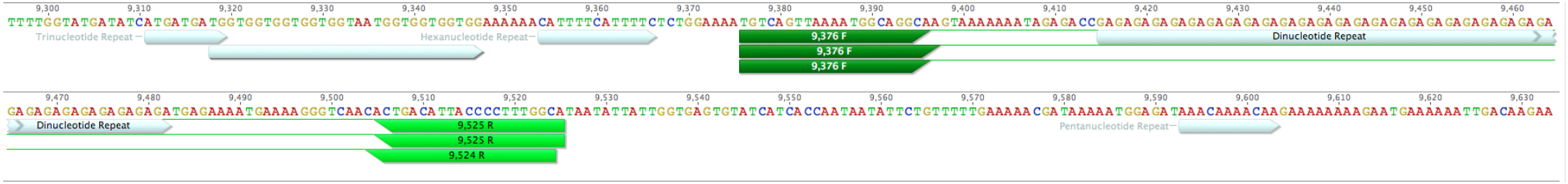
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
89	ASGP 01 011 543	AG	AG	34	68	9,415-9,482	66	100	F: TGTCAGTTAAAATGGCAGGCAA R: TGCCAAAGGGGTAATGTCAGT	59.0 59.6	150

Df MS 89 M13B F

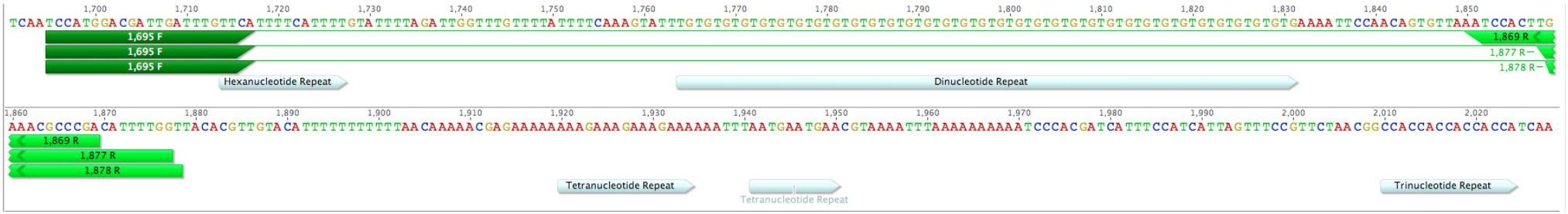
cactgcttagagcgatgcTGTCAGTTAAAATGGCAGGCAA

Df MS 89 R

gttTGCCAAAGGGGTAATGTCAGT



Dinucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
90	ASGP 01 004 796	AC	TG	34	68	1,764-1,831	66	100	F: TCCATGGACGATTGATTTGTTCA R1: TCGGGCGTTTCAAGTGGATT R2: CCAAAATGTCGGGCGTTTCA	58.7 60.3 59.7	 175 183
Two different combinations of primers.											
Df MS 90 M13B F cactgcttagagcgatgcTCCATGGACGATTGATTTGTTCA Df MS 90 R1 gttTCGGGCGTTTCAAGTGGATT Df MS 90 R2 gtttCCAAAATGTCGGGCGTTTCA											



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
91	ASGP 01 008 597	AG	AG	33	67	3,083-3,149	65	100	F1: TGGAGATATGAACACATAGCAACT F2: ACCACTCTATTGGAGATATGAACACA R: CCAAAATGTCGGGCGTTTCA	57.8 60.3 60.0	 185 193

Two different combinations of primers.

Df MS 91 M13B F1

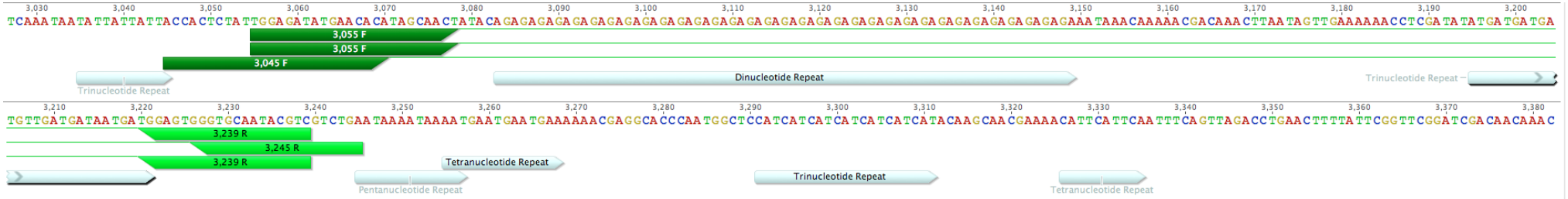
cactgcttagagcgatgcTGGAGATATGAACACATAGCAACT

Df MS 91 M13B F2

cactgcttagagcgatgcACCACTCTATTGGAGATATGAACACA

Df MS 91 R

gtttCCAAAATGTCGGGCGTTTCA



Dinucleotide repeats

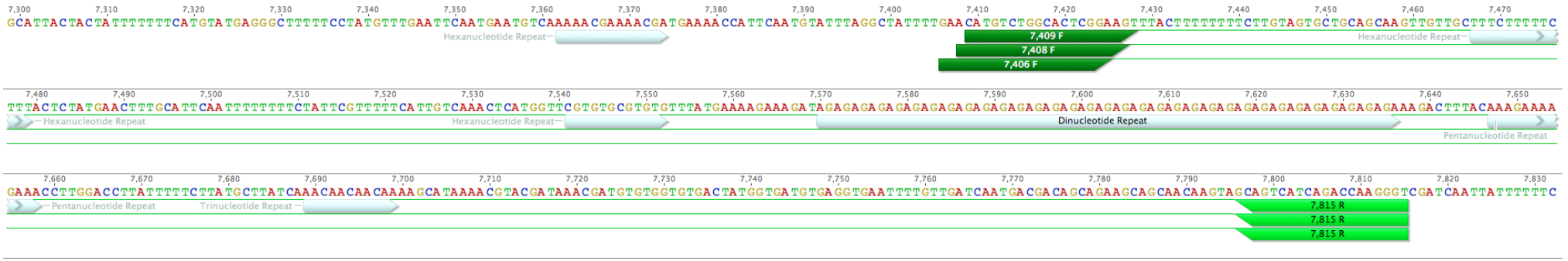
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
92	ASGP 01 005 489	AG	AC	33	67	7,570-7,636	65	100	F: CATGTCTGGCACTCGGAAGT R: ACCCTTGGTCTGATGACTGC	60.0 59.7	407

Df MS 92 M13B F

cactgcttagagcgatgCATGTCTGGCACTCGGAAGT

Df MS 92 R

gtttACCCTTGGTCTGATGACTGC



Dinucleotide repeats

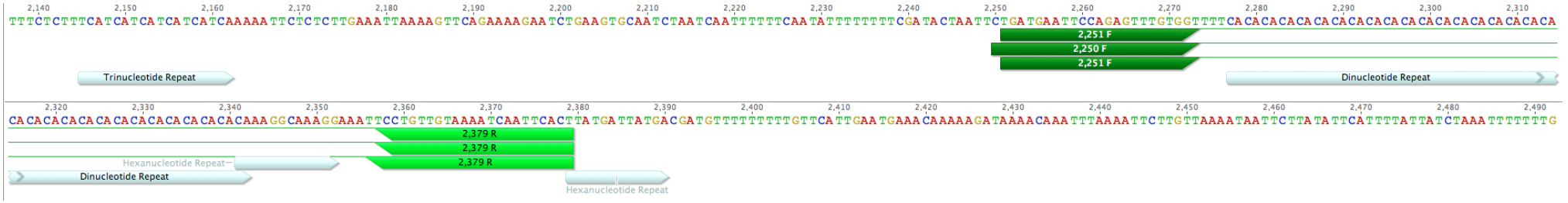
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
94	ASGP 01 002 265	AC	AC	33	66	2,277-2,342	64	100	F: TGATGAATTCCAGAGTTTGTGGT R: AGTGAATTGATTTTACAACAGGA	58.3 54.5	129

Df MS 94 M13B F

cactgcttagagcgatgcTGATGAATTCCAGAGTTTGTGGT

Df MS 94 R

gtttAGTGAATTGATTTTACAACAGGA



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
96	ASGP 01 000 062	AG	AG	33	66	1,688-1,753	64	100	F: TTCTCCTTCCATATTCAATGA R: TCGGGTTAGAATTGCAGACA	51.9 56.9	211

Df MS 96 M13B F

cactgcttagagcgatgcTTCTCCTTCCATATTCAATGA

Df MS 96 R

gttTCGGGTTAGAATTGCAGACA

Dinucleotide repeats

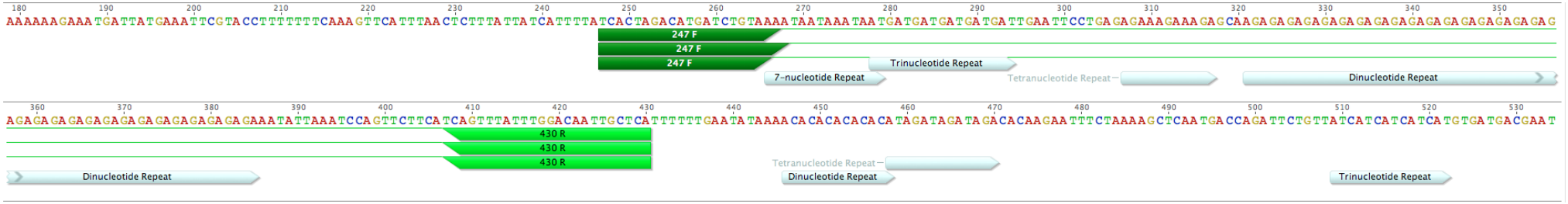
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
97	ASGP 01 009 025	AG	AG	32	65	321-385	63	100	F: TCACTAGACATGATCTGTAAAA R: TCGGGTTAGAATTGCAGACA	52.3 57.4	184

Df MS 97 M13B F

cactgcttagagcgatgcTCACTAGACATGATCTGTAAAA

Df MS 97 R

gttTCGGGTTAGAATTGCAGACA



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
98	ASGP 01 005 223	AC	TG	32	64	95-158	62	100	F: TTGCACACAGGGCAATTTGC R1: TCATCAACACAATTGGAAATGTTGA R2: TGGAAATGTTGAATTGAAACGA	60.5 58.2 55.1	 160 147

Two different combinations of primers.

Df MS 98 M13B F

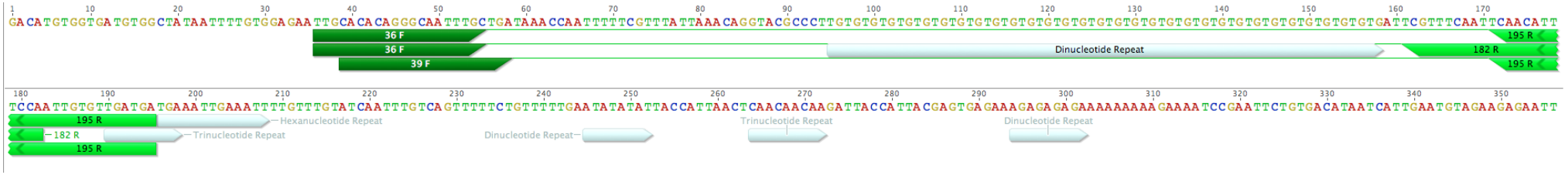
cactgcttagagcgatgcTTGCACACAGGGCAATTTGC

Df MS 98 R1

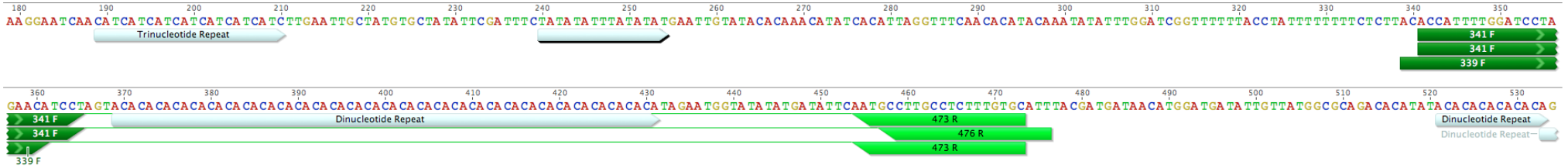
gttTCATCAACACAATTGGAAATGTTGA

Df MS 98 R2

gttTGGAAATGTTGAATTGAAACGA



Dinucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
99	ASGP 01 007 976	AC	AC	31	63	369-431	61	100	F: ACCATTTTGGATCCTAGAACATCCT R: GCACAAAGAGGCAAGGCATT	59.8 59.7	133
Df MS 99 M13B F cactgcttagagcgatgcACCATTTTGGATCCTAGAACATCCT Df MS 99 R gtttGCACAAAGAGGCAAGGCATT											



Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
100	ASGP 01 006 901	AG	AG	31	62	754-815	60	100	F: AGCCATCCATTCATCTCATCCA R: AGTTTCGAATCAAATACTGTATCCGT	59.3 59.0	150

Df MS 100 M13B F

cactgcttagagcgatgcAGCCATCCATTCATCTCATCCA

Df MS 100 R

gtttAGTTTCGAATCAAATACTGTATCCGT

Dinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
101	ASGP 01 010 696	AC	GT	59	118	5,091-5,208	116	100	F: GGTCGGTTGCTAGCGTGTAA R: TTTCATTCTTCGGCGCTTCA	60.4 58.4	319

Df MS 100 M13B F

cactgcttagagcgatgcGGTCGGTTGCTAGCGTGTAA

Df MS 100 R

gTTTCATTCTTCGGCGCTTCA

Trinucleotide repeats

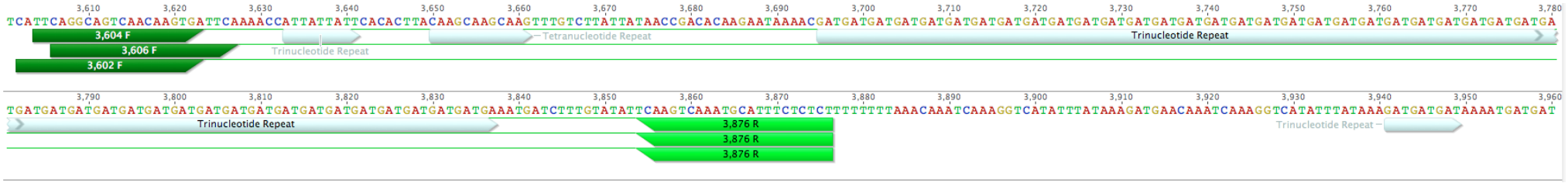
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
4	ASGP 01 009 178	ATC	GAT	47	143	3,695-3,837	140	100	F: TTCAGGCAGTCAACAAGTGA R: AGAGAGAAATGCATTTGACTTGA	57.3 56.5	273

Df MS 4 M13B F

cactgcttagagcgatgcTTCAGGCAGTCAACAAGTGA

Df MS 4 R

gtttAGAGAGAAATGCATTTGACTTGA



Trinucleotide repeats

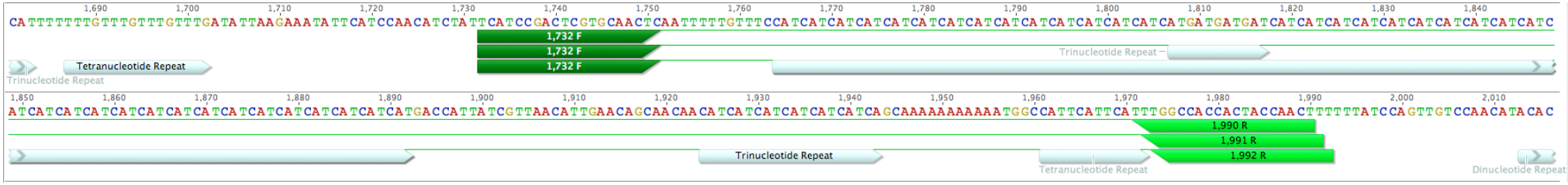
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
8	ASGP 01 011 072	ATC	CAT	43	129	1,764-1,892	108	98	F: TCATCCGACTCGTGCAACTC R: AGTTGGTAGTGGTGGCCAAA	60.0 59.4	259

Df MS 8 M13B F

cactgcttagagcgatgcTCATCCGACTCGTGCAACTC

Df MS 8 R

gtttAGTTGGTAGTGGTGGCCAAA



Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
15	ASGP 01 003 098	ATC	GAT	36	108	12,230-12,337	28	88	F1: TCGGATGATGGCGATGATGG F2: GGCGATGATGGGGAATGGAA R: TCTTCGGAGCTATTCTGCAACA	60.0 60.2 59.8	 263 254

Two different combinations of primers.

Df MS 15 M13B F1

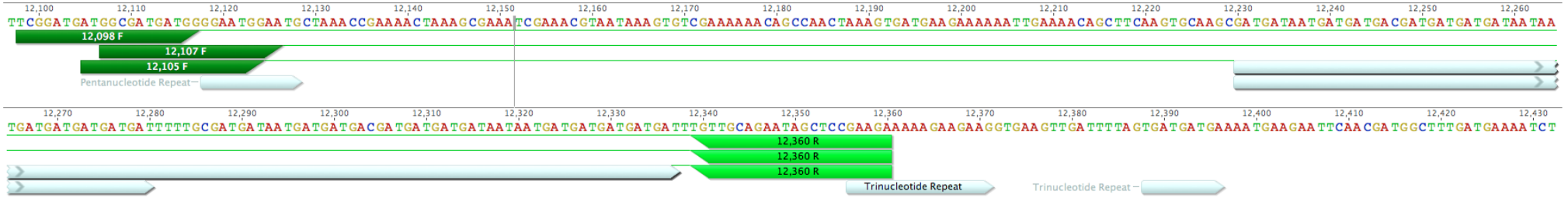
cactgcttagagcgatgcTCGGATGATGGCGATGATGG

Df MS 15 M13B F2

cactgcttagagcgatgcGGCGATGATGGGGAATGGAA

Df MS 15 R

gttTCTTCGGAGCTATTCTGCAACA



Trinucleotide repeats

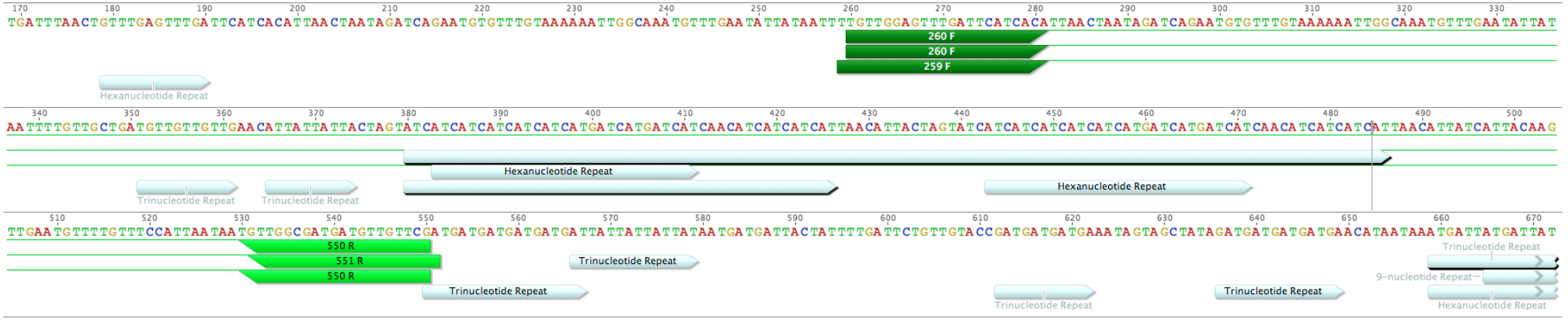
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
16	ASGP 01 007 696	ATC	ATC	35	107	308-486	28	88	F: TGTTGGAGTTTGATTCATCACA R: CGAACAACATCATCGCCAACA	56.2 59.8	291

Df MS 16 M13B F

actgcttagagcgatgcTGTTGGAGTTTGATTCATCACA

Df MS 16 R

gtttCGAACAACATCATCGCCAACA



Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
17	ASGP 01 003 851	AAC	GTT	33	103	19,761-19,861	28	88	F: TCATTTTTAGATTTTGTCGATGA R: TCGTTTTGATTATGCAGCACA	52.8 56.8	224

Df MS 17 M13B F

cactgcttagagcgatgcTCATTTTTAGATTTTGTCGATGA

Df MS 17 R

gtttTCGTTTTGATTATGCAGCACA

Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
20	ASGP 01 001 217	ATC	ATC	32	97	1,020-1,116	46	92	F1: AACTCAATGTCCAGCAGCGA F2: TGTCCAGCAGCGATGACATT R: TGCTGATGCTGATGCTGCTA	60.0 60.0 59.8	 358 351

Two different combinations of primers.

Df MS 20 M13B F1

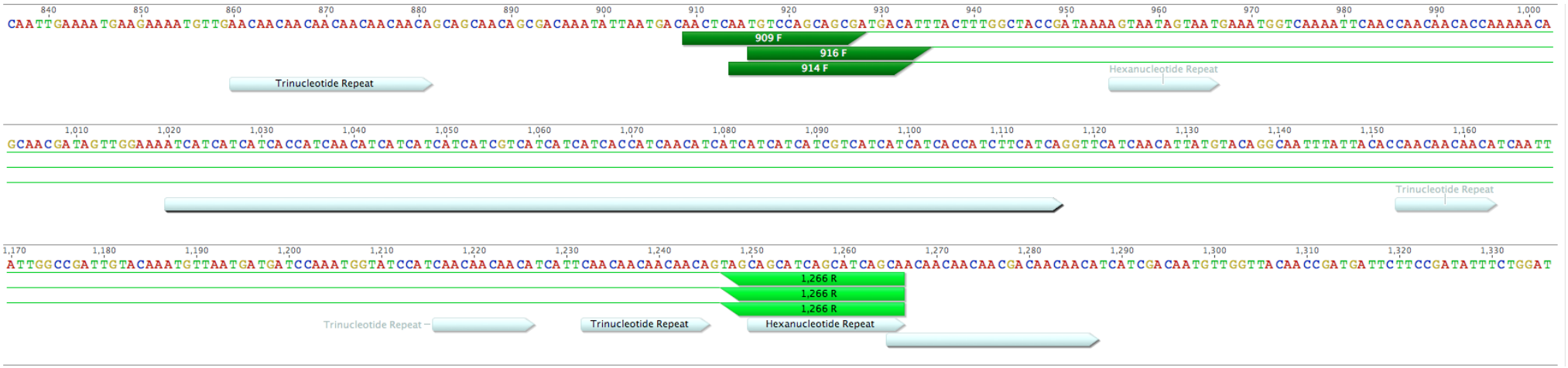
cactgcttagagcgatgcAACTCAATGTCCAGCAGCGA

Df MS 20 M13B F2

cactgcttagagcgatgcTGTCCAGCAGCGATGACATT

Df MS 20 R

gttTGCTGATGCTGATGCTGCTA



Trinucleotide repeats

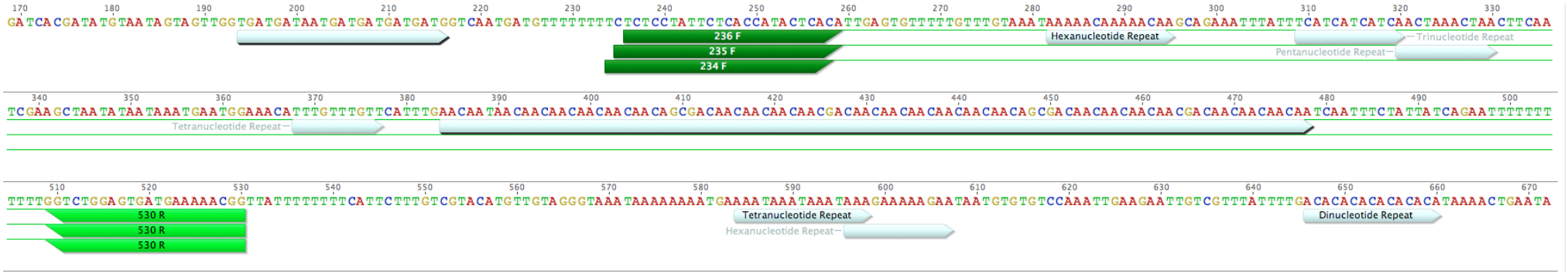
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
21	ASGP 01 007 928	AAC	AAC	31	95	384-478	50	93	F: TCTCCTATTCTCACCATACTCACA R: CCGTTTTTCATCACTCCAGACC	58.2 59.5	295

Df MS 21 M13B F

cactgcttagagcgatgcTCTCCTATTCTCACCATACTCACA

Df MS 21 R

gtttCCGTTTTTCATCACTCCAGACC



Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	%	Tm ˚C	Prod. bp
22	ASGP 01 002 137	ATC	GAT	31	94	9,452-9,545	43	91	F: CCACCATCAACATCACCATCG R1: TGATGATGATTGCGTTTGTTTCA R2: TGAATGATGATGATTGCGTTTGT	59.3 57.9 57.7	 432 436

Two different combinations of primers.

Df MS 22 M13B F

cactgcttagagcgatgCCACCATCAACATCACCATCG

Df MS 22 R1

gttTGATGATGATTGCGTTTGTTTCA

Df MS 22 R2

gttTGAATGATGATGATTGCGTTTGT

Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
23	ASGP 01 011 403	ATC	ATC	31	93	33-125	60	95	F: TCATTGTTGTCGTTGTTTGTTCA R: TCGTGGAAACAAATATCGCAACC	58.0 60.1	271

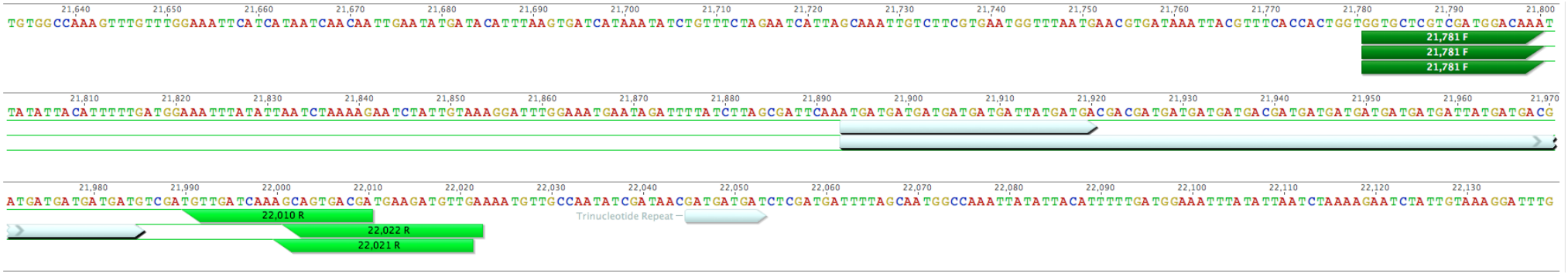
Df MS 23 M13B F

cactgcttagagcgatgcTCATTGTTGTCGTTGTTTGTTCA

Df MS 23 R

gttTCGTGGAAACAAATATCGCAACC

Trinucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**%Primers**	**Tm ˚C**	**Prod. bp**
24	ASGP 01 007 287	ATC	ATG	31	93	21,893-21,985	54	94	F: GGTGCTCGTCGATGGACAAA R1: TCGTCACTGCTTTGATCAACA R2: TCAACATCTTCATCGTCACTGC	60.7 58.2 59.0	 230 242
Two different combinations of primers.											
Df MS 24 M13B F cactgcttagagcgatgcGGTGCTCGTCGATGGACAAA Df MS 24 R1 gttTCGTCACTGCTTTGATCAACA Df MS 24 R2 gttTCAACATCTTCATCGTCACTGC											



Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primes	Tm ˚C	Prod. bp
26	ASGP 01 004 775	ATC	TCA	30	91	942-1,033	36	90	F: TCCGTGATGATATGATGTTCCA R: TGAAAAGAATTTCGTCAACAACA	57.0 55.8	273

Df MS 26 M13B F

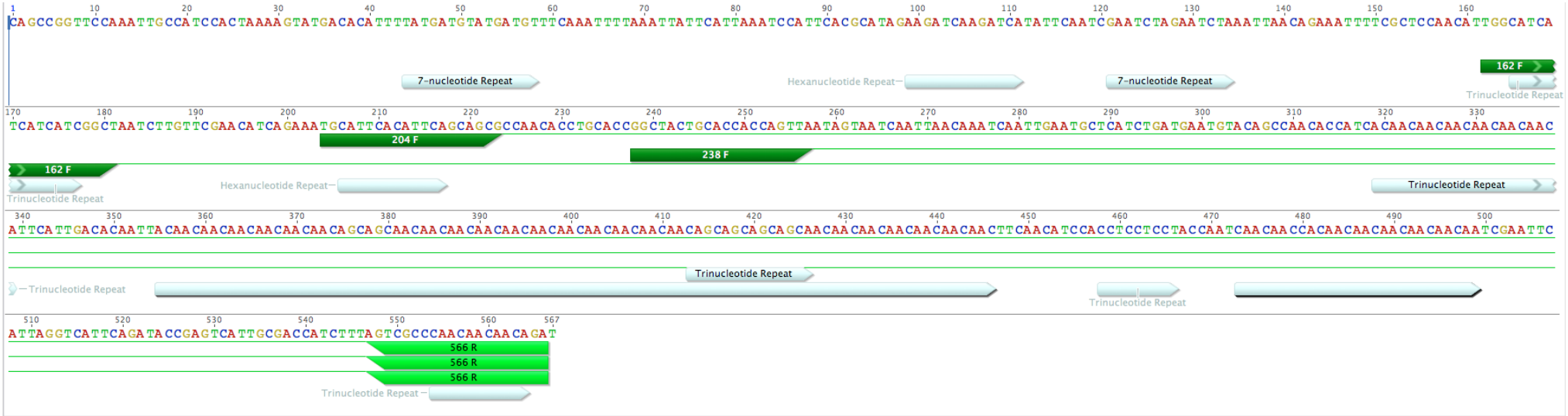
cactgcttagagcgatgcTCCGTGATGATATGATGTTCCA

Df MS 26 R

gttTGAAAAGAATTTCGTCAACAACA

Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
27	ASGP 01 003 496	AAC	ACA	30	92	355-446	53	93	F1: TGCATTCACATTCAGCAGCG F2: GGCTACTGCACCACCAGTTA R: TCTGTTGTTGTTGGGCGACT	59.8 59.7 60.1	 363 329
Two different combinations of primers.											
Df MS 27 M13B F1 cactgcttagagcgaTGCATTCACATTCAGCAGCG Df MS 27 M13B F2 cactgcttagagcgatgcGGCTACTGCACCACCAGTTA Df MS 27 R gtttTCTGTTGTTGTTGGGCGACT											



Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
28	ASGP 01 005 589	ATC	CAT	30	90	203-292	30	89	F: CATCATCAACATCGGCTGCG R1: GAGCAAATCTTGATCAATTAA R2: ATTGAGCAAATCTTGATCAATTAA	60.0 50.0 53.6	 251 254

Two different combinations of primers.

Df MS 28 M13B F

cactgcttagagcgatgCATCATCAACATCGGCTGCG

Df MS 28 R1

gtttGAGCAAATCTTGATCAATTAA

Df MS 28 R2

gtttATTGAGCAAATCTTGATCAATTAA

Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
30	ASGP 01 009 299	AAC	CAA	29	89	973-1061	50	93	F1: ACATGAACGAAGACGACGAGA F2: ACAACATGAACGAAGACGACG R: ACCCGTTGTCCATTAACTTTCA	59.5 59.2 58.2	 417 420

Two different combinations of primers.

Df MS 30 M13B F1

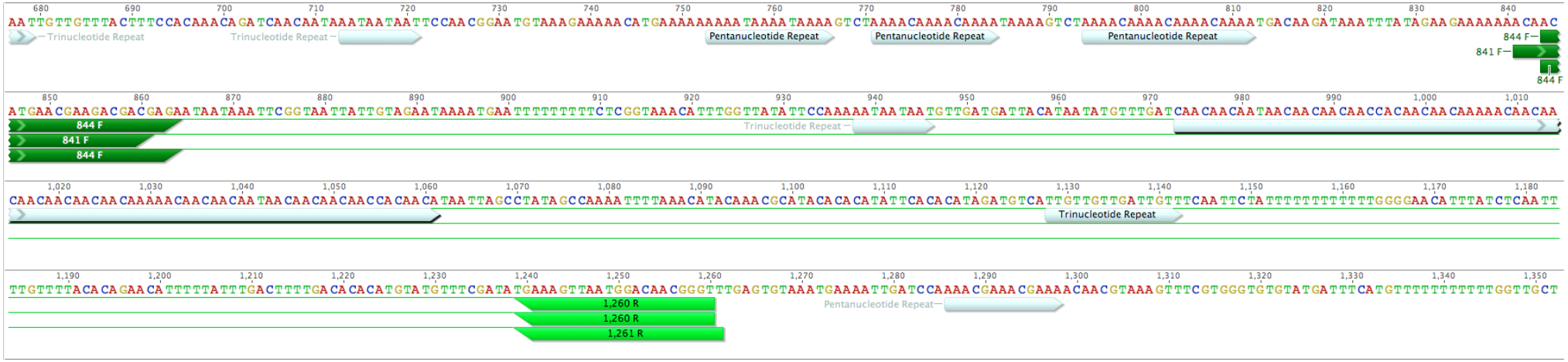
cactgcttagagcgatgcACATGAACGAAGACGACGAGA

Df MS 30 M13B F2

cactgcttagagcgatgcACAACATGAACGAAGACGACG

Df MS 30 R

gtttACCCGTTGTCCATTAACTTTCA



Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
31a	ASGP 01 005 955	AAC	CAA	29	88	4,947-5,036	33	90	F: ATGCGGGCGATCAATATGGT R1: TCTTGTGAATCCGATTGTGCT R2: TCCGATTGTGCTAATTGTTGCT	60.0 57.9 58.9	 314 332
Two different combinations of primers.											
Df MS 29 M13B F cactgcttagagcgATGCGGGCGATCAATATGGT Df MS 29 R1 gttTCTTGTGAATCCGATTGTGCT Df MS 29 R2 gttTCCGATTGTGCTAATTGTTGCT											
31b	ASGP 01 005 955	AAC	CAA	29	88	4,947-5,036	33	90	F: ATGCGGGCGATCAATATGGT R3: AGGCAGGTTTATGATGAGCA	56.9	473
Df MS 29 R3 gtttAGGCAGGTTTATGATGAGCA These are two related but different microsatellites, the first one with two different primer combinations, the second one with one primer combination. AGGCAGGTTTATGATGAGCA											

Trinucleotide repeats

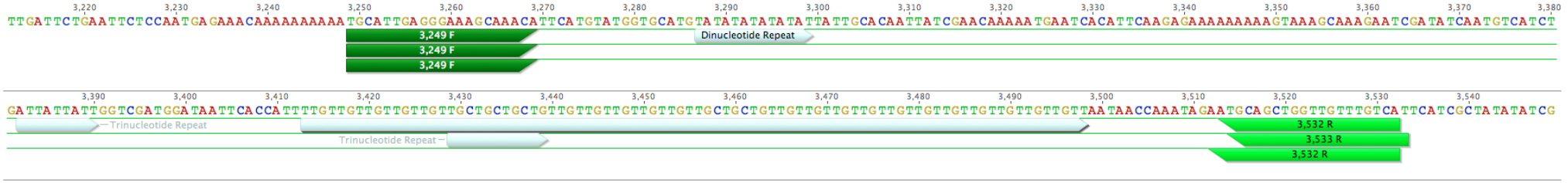
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
33	ASGP 01 010 464	AAC	TTG	43	86	3,413-3,498	53	94	F: TGCATTGAGGGAAAGCAAACA R: TGACAAACAACCAGCTGCAT	59.0 58.6	284

Df MS 33 M13B F

cactgcttagagcgatgcTGCATTGAGGGAAAGCAAACA

Df MS 33 R

gttTGACAAACAACCAGCTGCAT



Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
36	ASGP 01 006 735	ATC	TGA	28	86	1,164-1,248	41	92	F: ACAAGAATCGAATCTTCTCCA R1: TCATTGATTTCACGATCGATTTGA R2: TCATTTCATTGATTTCACGATCGA	54.7 57.3 57.3	 202 207

Two different combinations of primers.

Df MS 36 M13B F

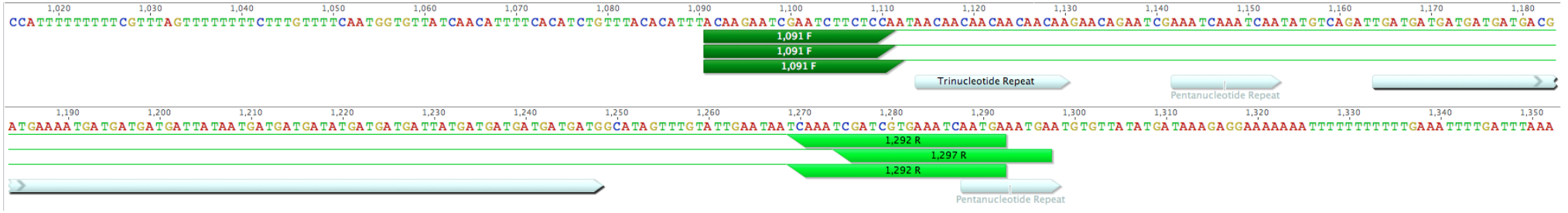
cactgcttagagcgatgcACAAGAATCGAATCTTCTCCA

Df MS 36 R1

gttTCATTGATTTCACGATCGATTTGA

Df MS 36 R2

gttTCATTTCATTGATTTCACGATCGA



Trinucleotide repeats

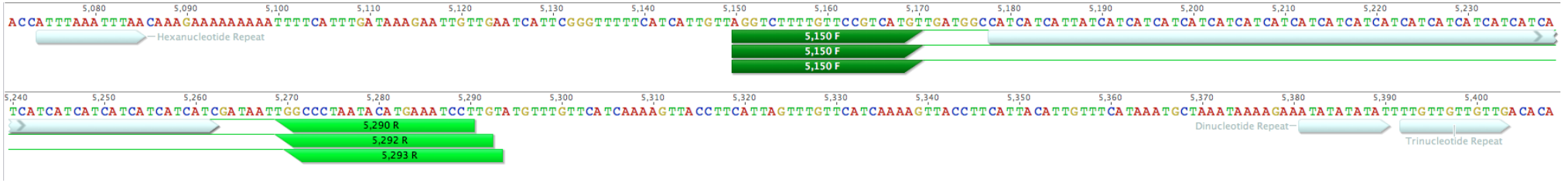
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
37	ASGP 01 005 080	ATC	CAT	28	85	5,178-5,262	76	99	F: AGGTCTTTTGTTCCGTCATGT R: ACAAGGATTTCATGTATTAGGGCC	57.8 58.9	144

Df MS 37 M13B F

cactgcttagagcgatgcAGGTCTTTTGTTCCGTCATGT

Df MS 37 R

gtttACAAGGATTTCATGTATTAGGGCC



Trinucleotide repeats

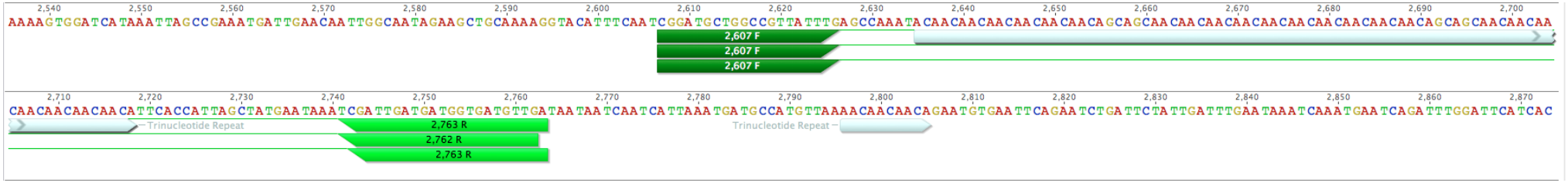
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
38	ASGP 01 010 749	AAC	ACA	28	84	2,635-2,718	57	95	F: CGGATGCTGGCCGTTATTTG R: TCAACATCACCATCATCAATCGA	60.0 58.2	157

Df MS 38 M13B F

cactgcttagagcgatgCGGATGCTGGCCGTTATTTG

Df MS 38 R

gttTCAACATCACCATCATCAATCGA



Trinucleotide repeats

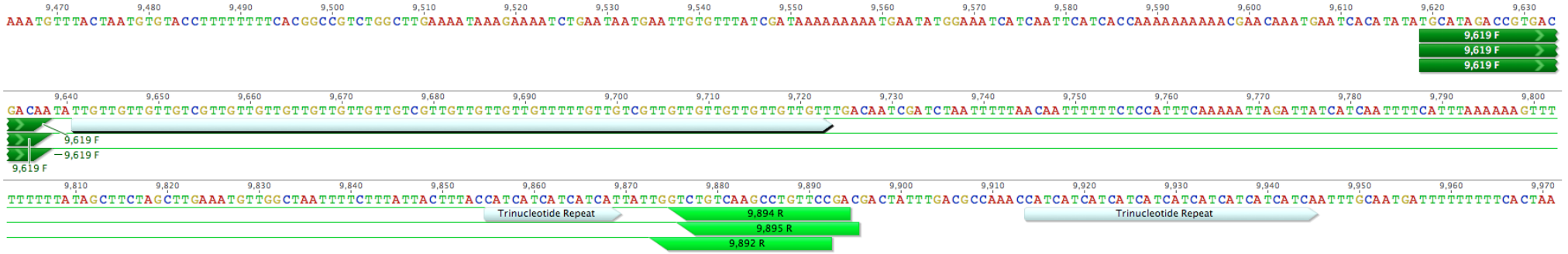
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
42	ASGP 01 010 203	AAC	TTG	27	83	9,641-9,723	56	95	F: TGCATAGACCGTGACGACAA R: TCGGAACAGGCTTGACAGAC	59.4 60.0	276

Df MS 42 M13B F

cactgcttagagcgaTGCATAGACCGTGACGACAA

Df MS 42 R

gttTCGGAACAGGCTTGACAGAC



Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
54a	ASGP 01 010 207	ATC	ATC	27	82	13,790-13,871	43	93	F: GCCATTCGCAAATTTTCGTGAC R1: GCTCTTGTTGCTTTTGTGGCT	59.5 60.2	302
	Df MS 45 M13B F cactgcttagagcgaGCCATTCGCAAATTTTCGTGAC Df MS 45 R1 gtttGCTCTTGTTGCTTTTGTGGCT										
54b	ASGP 01 010 207	ATC	ATC	27	82	13,790-13,871	43	93	F: GCCATTCGCAAATTTTCGTGAC R2: GCCAACCAGTTTGCTGTTGT	59.5 59.8	434
	Df MS 42 R2 gtttGCCAACCAGTTTGCTGTTGT										

These are two related but different microsatellites.

Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
47	ASGP 01 008 257	AAC	TTG	27	81	9,824-9,904	42	93	F: TGTGGTTCAATCCAACATTCTGT R: TGGGTGATCATCAACATCGTCA	58.9 59.8	220

Df MS 47 M13B F

cactgcttagagcgatgcTGTGGTTCAATCCAACATTCTGT

Df MS 47 R

gttTGGGTGATCATCAACATCGTCA

Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
48	ASGP 01 006 650	AAC	TTG	27	81	346-426	42	93	F1: TGACTGGGGAGGTGGAAAGA F2: TTTGGCCAATTGACTGGGGA R: AGATAGAATCCGGACCAGAA	60.1 59.8 55.0	 175 185

Two different combinations of primers.

Df MS 48 M13B F1

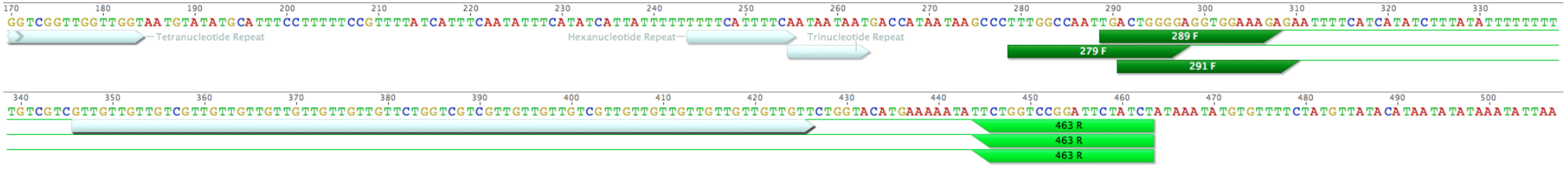
cactgcttagagcgatgcTGACTGGGGAGGTGGAAAGA

Df MS 48 M13B F2

cactgcttagagcgatgcTTTGGCCAATTGACTGGGGA

Df MS 48 R

gtttAGATAGAATCCGGACCAGAA



Trinucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
49	ASGP 01 005 718	ATC	TGA	26	80	611-691	24	89	F: CGTACACGTTTTGTTGGCGA R1: GCAGCATCGGCATCATCATC R2: ATCAGCAGCATCGGCATCAT	59.7 59.8 60.3	 293 297

Two different combinations of primers.

Df MS 49 M13B F

cactgcttagagcgatgCGTACACGTTTTGTTGGCGA

Df MS 49 R1

gtttGCAGCATCGGCATCATCATC

Df MS 49 R2

gtttATCAGCAGCATCGGCATCAT

Trinucleotide repeats

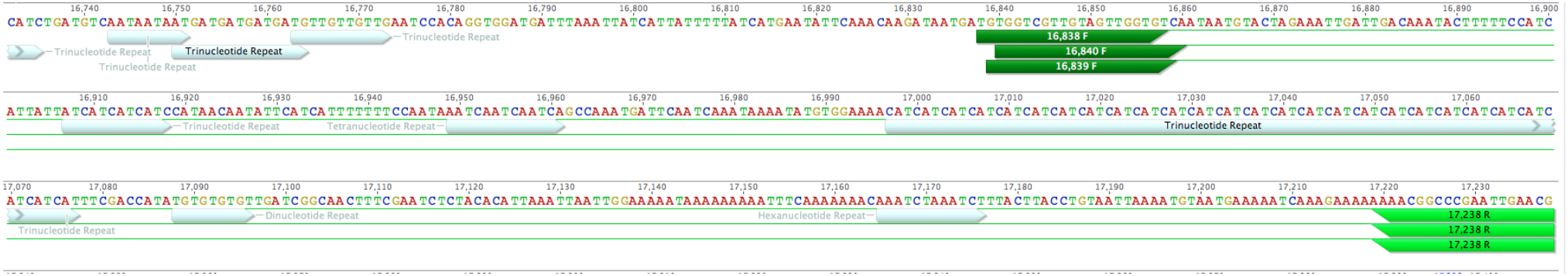
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
50	ASGP 01 005 604	ATC	CAT	27	81	16,997-17,077	78	100	F: TGTGGTCGTTGTAGTTGGTGT R: CGTTCAATTCGGGCCGTTTT	59.8 60.0	401

Df MS 50 M13B F

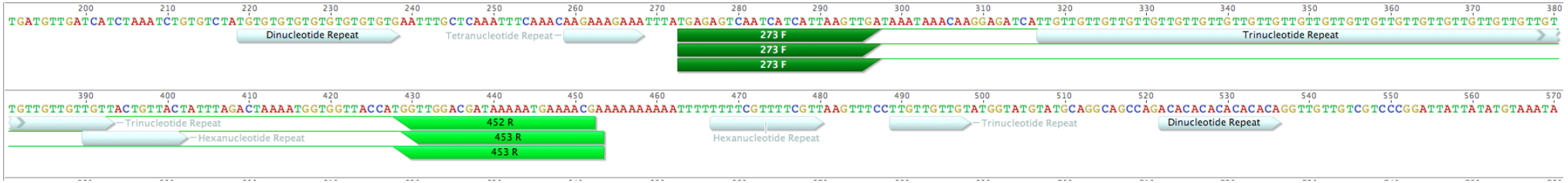
cactgcttagagcgatgcTGTGGTCGTTGTAGTTGGTGT

Df MS 50 R

gtttCGTTCAATTCGGGCCGTTTT



Trinucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
78	ASGP 01 006 439	AAC	TTG	25	**77**	317-3F	74	100	F: TGAGAGTCAATCATCATTAAGTTGA R: CGTTTTCATTTTTATCGTCCAACCA	56.3 59.1	180
Df MS 78 M13B F cactgcttagagcgatgcTGAGAGTCAATCATCATTAAGTTGA Df MS 78 R tttCGTTTTCATTTTTATCGTCCAACCA											



Tetranucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
52	ASGP 01 009 773	ATCC	ATGG	19	75	18,300-18,376	49	95	F: CACACAAGTGGGGAAAGGAGA R: AGAGAGCAAAAGTGAGAGGACA	59.9 59.0	184

Df MS 52 M13B F

cactgcttagagcgatgCACACAAGTGGGGAAAGGAGA

Df MS 52 R

gtttAGAGAGCAAAAGTGAGAGGACA

Tetranucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
59	ASGP 01 004 575	AATG	AATG	18	73	2,611-2,686	26	89	F1: TGGATGGGTGGAAATGGTGG F2: ATTGGGGAATGGATGGGTGG R: GGGGGTGTCTATTTCTCTCT	60.0 55.0 55.6	 155 168

Two different combinations of primers.

Df MS 59 M13B F1

cactgcttagagcgatgcTGGATGGGTGGAAATGGTGG

Df MS 59 M13B F2

cactgcttagagcgatgcATTGGGGAATGGATGGGTGG

Df MS 59 R

gtttGGGGGTGTCTATTTCTCTCT

Tetranucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
60	ASGP 01 002 694	AACC	AACC	11	44	3,553-3,596	40	100	F: AACCGACTAACCAGCCAACC R: AGTAGCGGCAAAACTCCACA	60.3 59.9	171

Df MS 60 M13B F
cactgcttagagcgatgcAACCGACTAACCAGCCAACC
Df MS 60 R
gtttAGTAGCGGCAAAACTCCACA

Tetranucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
64	ASGP 01 002 201	AGAT	TCTA	16	67	4,278-4,343	28	91	F: AGCTTAGAAATCAACATTTGCCA R: TGCAACAGAATTGAAAGTGGGA	57.3 58.7	204

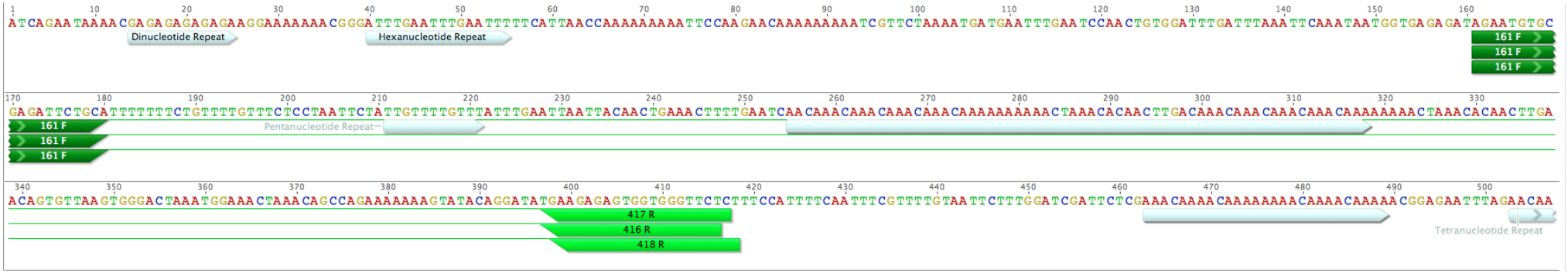
Df MS 64 M13B F

cactgcttagagcgatgcAGCTTAGAAATCAACATTTGCCA

Df MS 64 R

gttTGCAACAGAATTGAAAGTGGGA

Tetranucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
66	ASGP 01 004 405	AAAC	AAAC	15	62	255-318	19	89	F: AGAATGTGCGAGATTCTGCA R: GAGAACCCACCACTCTCTTCA	57.6 59.0	257
Df MS 66 M13B F cactgcttagagcgatgcAGAATGTGCGAGATTCTGCA Df MS 66 R gtttGAGAACCCACCACTCTCTTCA											



Tetranucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
67	ASGP 01 009 275	AACC	AACC	15	63	5,698-5,760	41	95	F: AGCTACAACAACAGTGAAACACA R: TATTGATGGCCTGGCGGTTT	59.0 60.0	183
Df MS 67 M13B F cactgcttagagcgatgcAGCTACAACAACAGTGAAACACA Df MS 67 R gttTATTGATGGCCTGGCGGTTT											

Tetranucleotide repeats

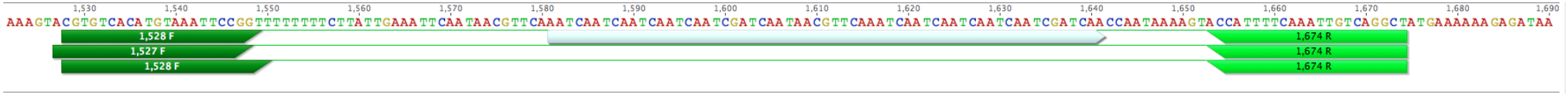
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
71	ASGP 01 002 015	AATC	AATC	15	62	1,581-1,641	17	89	F: CGTGTCACATGTAAATTCCGGT R: AGCCTGACAATTTGAAAATGGT	59.3 57.0	147

Df MS 71 M13B F

cactgcttagagcgatgCGTGTCACATGTAAATTCCGGT

Df MS 71 R

gtttAGCCTGACAATTTGAAAATGGT



Tetranucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
73	ASGP 01 008 213	AAAC	AAAC	14	59	3,938-3,997	21	90	F: ACAGTGAGATAAAGAATCCAAGA R1: TCTTGTCTAGCTTCATCATCTTCA R2: TGTCTAGCTTCATCATCTTCATTCA	55.1 57.5 58.1	 181 178

Two different combinations of primers.

Df MS 73 M13B F

cactgcttagagcgatgcACAGTGAGATAAAGAATCCAAGA

Df MS 73 R1

gttTCTTGTCTAGCTTCATCATCTTCA

Df MS 73 R2

gttTGTCTAGCTTCATCATCTTCATTCA

Pentanucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
58	ASGP 01 010 181	AAAAC	TTTTG	14	71	1,581-1,641	14	87	F: TCGTTACAGTCGTTGTTCAACT R: ACGGATGAAAAATCTGCCTGA	58.2 52.9	341

Df MS 58 M13B F

cactgcttagagcgatgcTCGTTACAGTCGTTGTTCAACT

Df MS 58 R

gtttACGGATGAAAAATCTGCCTGA

Pentanucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
69	ASGP 01 007 547	AAATT	TTTAA	11	58	892-957	14	88	F1: CTGTATTGTGCACGTGCTAA F2: TGTGCACGTGCTAAATTTCT R: TGTTCAAACCTAGACTATTCTGT	56.5 56.3 55.0	 210 204

Two different combinations of primers.

Df MS 69 M13B F1

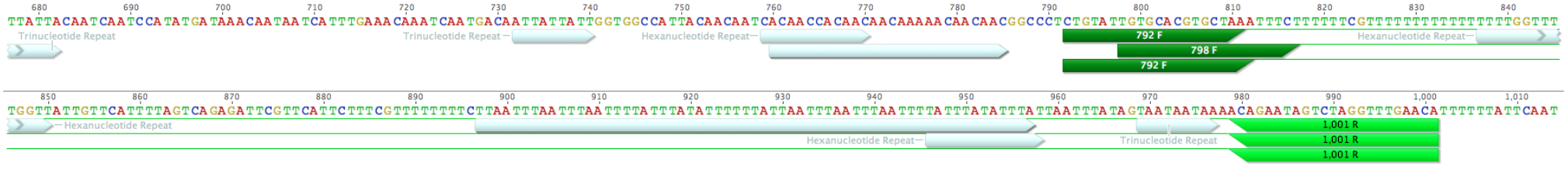
cactgcttagagcgatgCTGTATTGTGCACGTGCTAA

Df MS 69 M13B F2

cactgcttagagcgatgcTGTGCACGTGCTAAATTTCT

Df MS 69 R

gttTGTTCAAACCTAGACTATTCTGT



Pentanucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
75	ASGP 01 002 894	AACAT	ATGTT	12	60	11,477-11,536	43	97	F: AGTAAGTTTTCAATCTTGGAA R: TGCGACAACGATGACGATGA	50.9 60.0	222

Df MS 75 M13B F

cactgcttagagcgatgcAGTAAGTTTTCAATCTTGGAA

Df MS 75 R

gttTGCGACAACGATGACGATGA

Pentanucleotide repeats

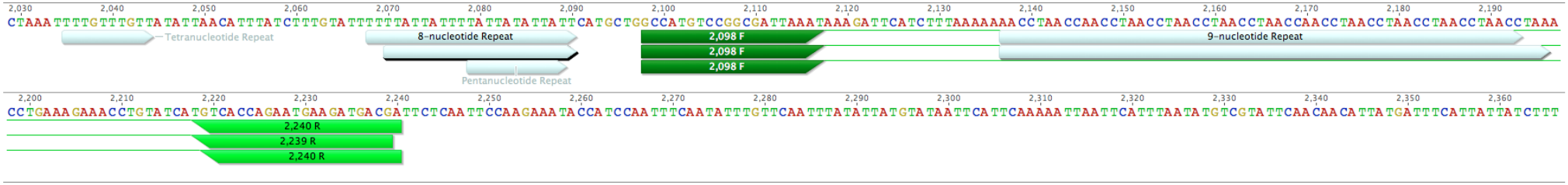
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
76	ASGP 01 001 333	AACCT	AACCT	12	62	2,137-2,196	45	97	F: GCCATGTCCGGCGATTAAAT R: TCGTCATCTTCATTCTGGTGACA	59.0 59.7	143

Df MS 76 M13B F

cactgcttagagcgatGCCATGTCCGGCGATTAAAT

Df MS 76 R

gttTCGTCATCTTCATTCTGGTGACA



Hexanucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
53	ASGP 01 007 155	AATCAT	TATGAT	13	83	1,115-1,197	24	89	F: TCAAAATCAGCATATCATGATGGT R1: AGATGTGCGAACATTTTAACA R2: TTCATAAAGATGTGCGAACA	56.8 54.4 53.2	 133 140

Two different combinations of primers.

Df MS 53 M13B F

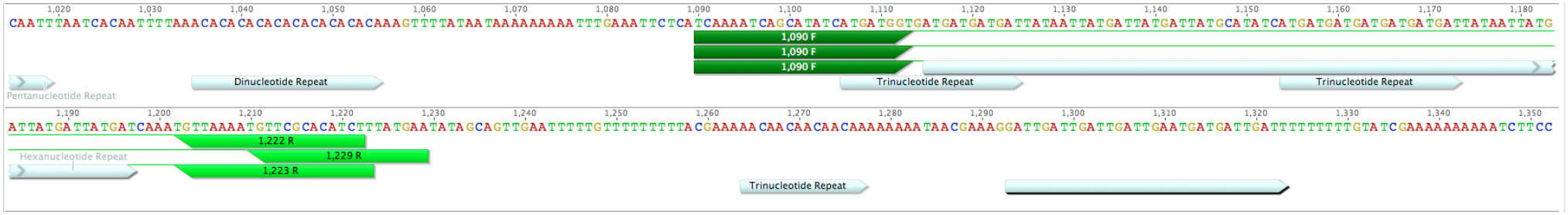
cactgcttagagcgatgcTCAAAATCAGCATATCATGATGGT

Df MS 53 R1

gtttAGATGTGCGAACATTTTAACA

Df MS 53 R2

gtTTCATAAAGATGTGCGAACA



Hexanucleotide repeats

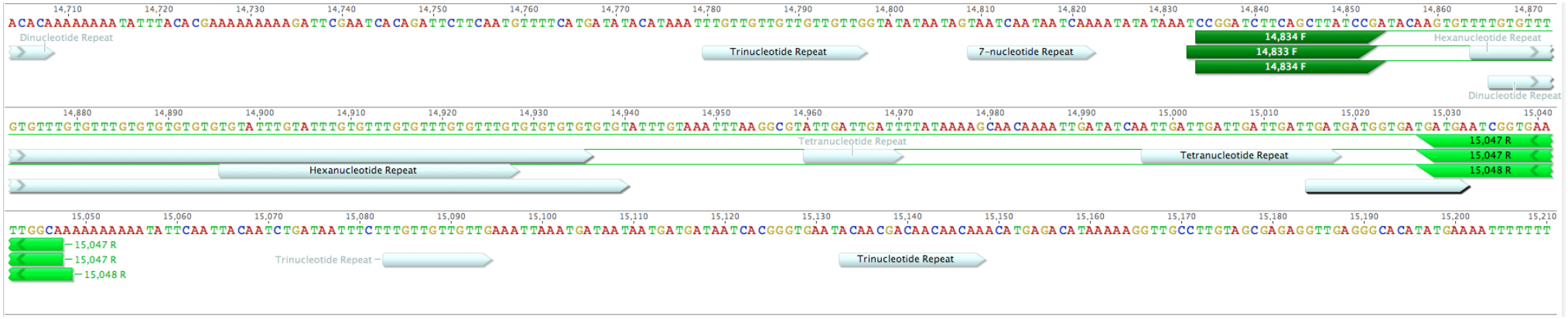
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
56	ASGP 01 007 389	AAACAC	TTTGTG	12	73	14,864-14,936	37	93	F: CCGGATCTTCAGCTTATCCGA R: TGCCAATTCACCGATTCATCA	59.4 58.3	214

Df MS 56 M13B F

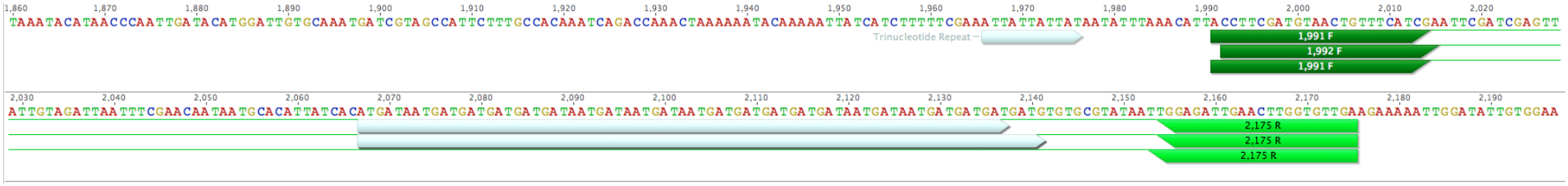
cactgcttagagcgatgCCGGATCTTCAGCTTATCCGA

Df MS 56 R

gttTGCCAATTCACCGATTCATCA



Hexanucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
57	ASGP 01 005 245	AATGAT	AATGAT	11	71	2,067-2,137	35	93	F: ACCTTCGATGTAACTGTTTCATCG R: TCAACACCAAGTTCAATCTCCA	59.4 57.8	185
Df MS 57 M13B F cactgcttagagcgatgcACCTTCGATGTAACTGTTTCATCG Df MS 57 R gttTCAACACCAAGTTCAATCTCCA											



The hexanucleotide repeat has 93.0 % perfection, while the dinucleotide repeat has 92.0 % perfection, therefore it is treated as a hexanucleotide repeat.

Hexanucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
61	ASGP 01 006 437	AAAAAC	TTTTTG	11	66	447-511	30	92	F: ACCCTCTAGCTATATGGCGA R1: GCTGCAAATTGTTTGGTTTTGGT R2: TGAGCTGCAAATTGTTTGGT	57.1 59.9 56.7	 177 180

Two different combinations of primers.

Df MS 61 M13B F

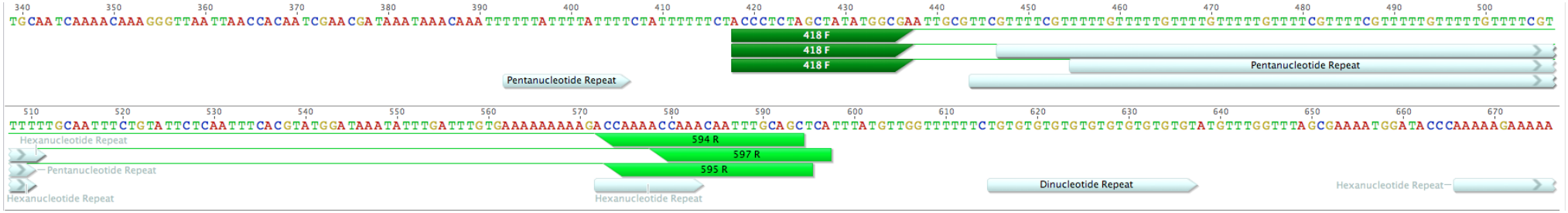
cactgcttagagcgatgcACCCTCTAGCTATATGGCGA

Df MS 61 R1

gtttGCTGCAAATTGTTTGGTTTTGGT

Df MS 61 R2

gttTGAGCTGCAAATTGTTTGGT



Hexanucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
62	ASGP 01 007 001	AATATG	ATATTC	11	66	1,355-1,420	24	91	F: AAAGACTTGCAGCCGGATGA R1: GCGGCAACAACACCATCATC R2: TCAATGTCAGCAATGTCGGC	60.0 60.5 59.2	 211 265

Two different combinations of primers.

Df MS 62 M13B F

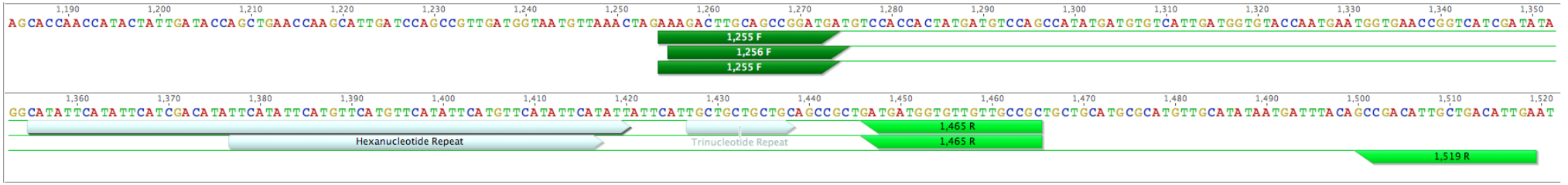
cactgcttagagcgatgcAAAGACTTGCAGCCGGATGA

Df MS 62 R1

gtttGCGGCAACAACACCATCATC

Df MS 62 R2

gttTCAATGTCAGCAATGTCGGC



Hexanucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
63	ASGP 01 002 229	AAAAAC	TTTTTG	11	68	10,584-10,649	27	91	F1: AAGCCGAAAGTTCACACGGT F2: CGAAAGTTCACACGGTCGAC R: TGGACGATGAAAAGAATGACAAACA	60.5 59.5 59.7	 218 277

Two different combinations of primers.

Df MS 63 M13B F1

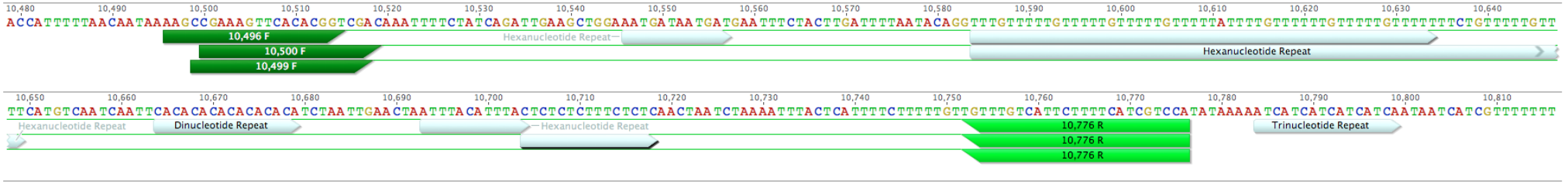
cactgcttagagcgatgcAAGCCGAAAGTTCACACGGT

Df MS 63 M13B F2

cactgcttagagcgatgcCGAAAGTTCACACGGTCGAC

Df MS 63 R

gttTGGACGATGAAAAGAATGACAAACA



Hexanucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
65	ASGP 01 006 437	AAAAAC	TTTTTG	10	66	447-511	30	92	F: ACCCTCTAGCTATATGGCGA R1: GCTGCAAATTGTTTGGTTTTGGT R2: TGAGCTGCAAATTGTTTGGT	57.1 59.9 56.7	 177 180

Two different combinations of primers.

Df MS 65 M13B F

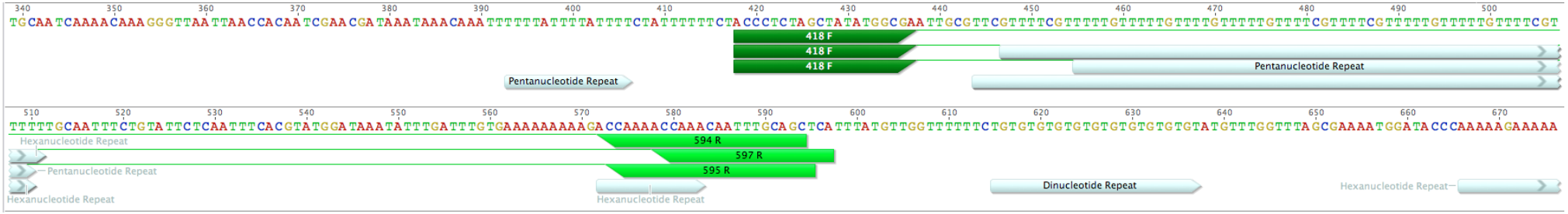
cactgcttagagcgatgcACCCTCTAGCTATATGGCGA

Df MS 65 R1

gtttGCTGCAAATTGTTTGGTTTTGGT

Df MS 65 R2

ttTGAGCTGCAAATTGTTTGGT



Hexanucleotide repeats

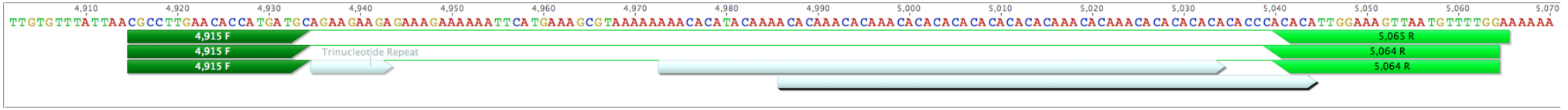
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
68	ASGP 01 004 678	AAACAC	AAACAC	10	61	4,973-5,034	21	90	F: CGCCTTGAACACCATGATGC R: TCCAAAACATTAACTTTCCAATGTGT	60.2 58.4	151

Df MS 68 M13B F

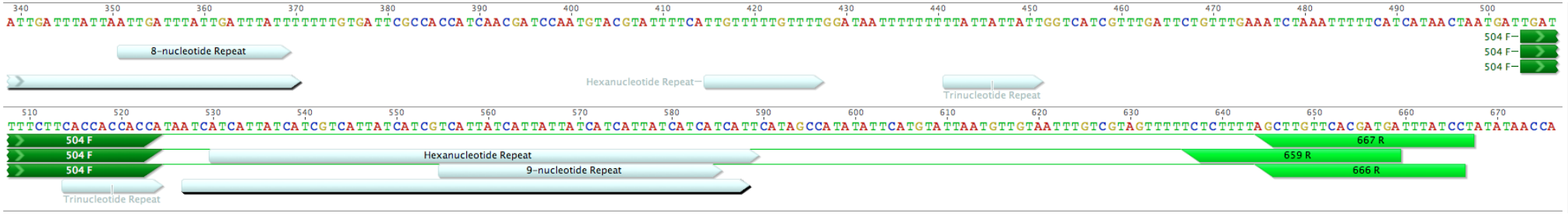
cactgcttagagcgatgcCGCCTTGAACACCATGATGC

Df MS 68 R

gttTCCAAAACATTAACTTTCCAATGTGT

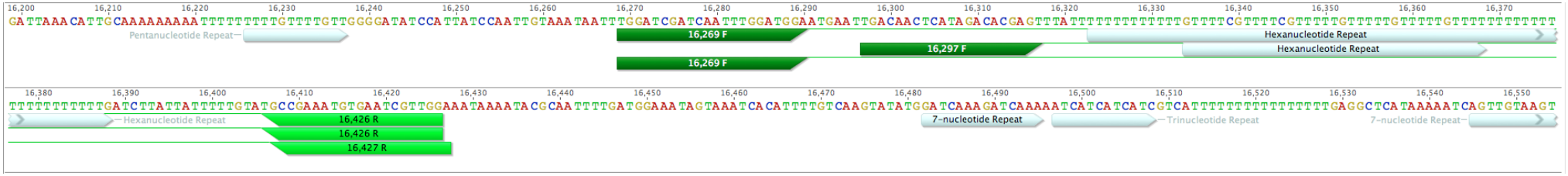


Hexanucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
74	ASGP 01 007 686	AATGAT	TTACAT	10	60	530-589	12	88	F: TGATTTTCTTCACCACCACCA R1: AGGATAAATCATCGTGAACAAGCT R2: TCATCGTGAACAAGCTAAAAGAGA	57.7 58.6 58.5	 164 156
Two different combinations of primers.											
Df MS 74 M13B F cactgcttagagcgatgcTGATTTTCTTCACCACCACCA Df MS 74 R1 gtttAGGATAAATCATCGTGAACAAGCT Df MS 74 R2 gttTCATCGTGAACAAGCTAAAAGAGA											



The hexanucleotide repeat has 88.3 % perfection, while the trinucleotide repeat has 87.0 % perfection, therefore it is treated as a hexanucleotide repeat.

Hexanucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
93	ASGP 01 002 343	AAAAAC	TTTTTG	11	66	16,323-16,388	24	91	F: TGGATCGATCAATTTGGATGGA R: CCAACGATTCACATTTCGGCA	57.6 59.8	158
Df MS 93 M13B F cactgcttagagcgatgcTGGATCGATCAATTTGGATGGA Df MS 93 R gtttCCAACGATTCACATTTCGGCA											



Hexanucleotide repeats

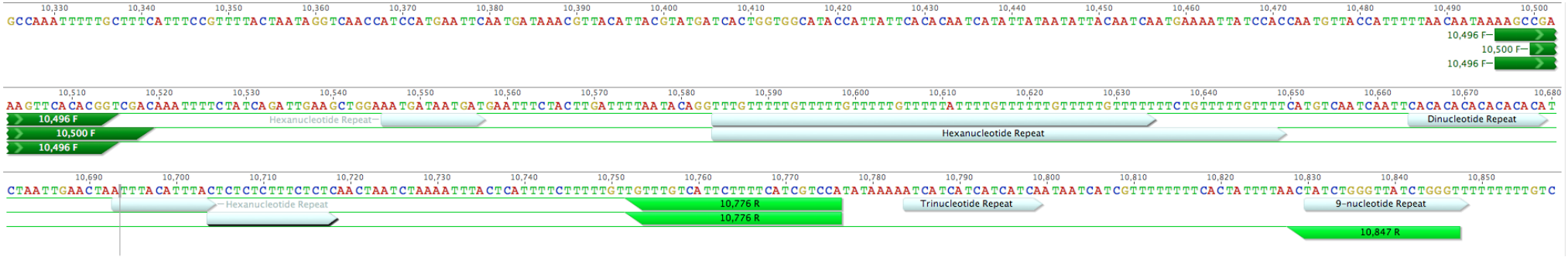
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
95	ASGP 01 002 229	AAAAAC	TTTTTG	11	68	10,584-10,649	27	91	F: AAGCCGAAAGTTCACACGGT R: TGGACGATGAAAAGAATGACAAACA	60.5 59.7	281

Df MS 95 M13B F

cactgcttagagcgatgcAAGCCGAAAGTTCACACGGT

Df MS 95 R

gttTGGACGATGAAAAGAATGACAAACA



Heptanucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
70	ASGP 01 006 491	AATCTAG	AATCTAG	9	64	2,361-2,421	21	91	F1: TGAGCCCAACACAACATTTT F2: AAAATGAGCCCAACACAACA R: TGTTTTGGCTTGAAATGGGA	56.3 56.3 56.0	 133 137

Two different combinations of primers.

Df MS 70 M13B F1

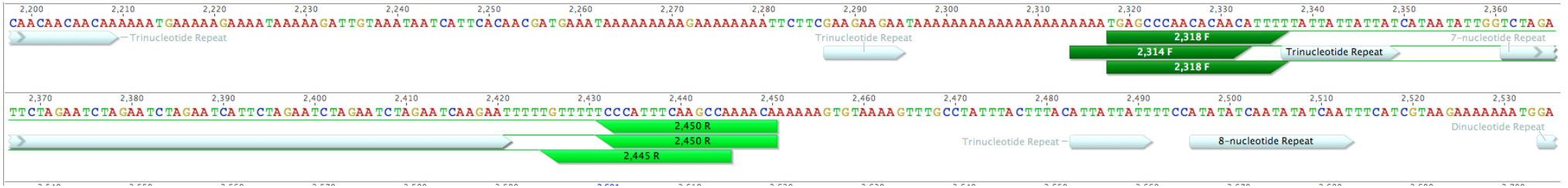
cactgcttagagcgatgcTGAGCCCAACACAACATTTT

Df MS 70 M13B F2

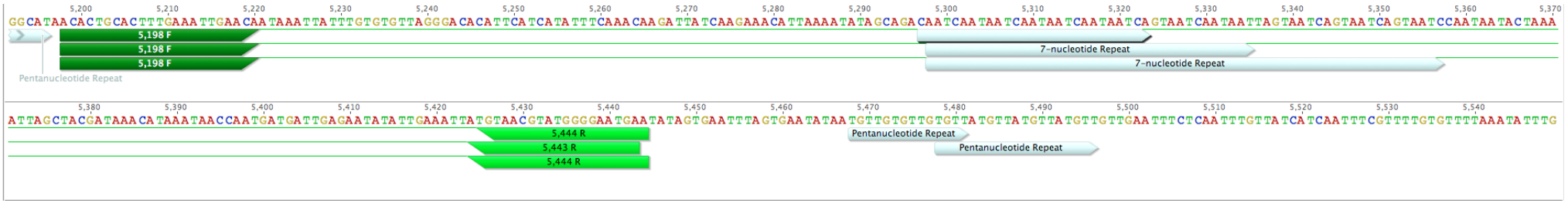
cactgcttagagcgatgcAAAATGAGCCCAACACAACA

Df MS 70 R

gttTGTTTTGGCTTGAAATGGGA



Heptanucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
72	ASGP 01 008 630	AATCAGT	AATCAGT	8	60	5,298-5,357	23	92	F: ACACTGCACTTTGAAATTGAACA R: TTCATTCCCCATACGTTACA	54.1	247
Df MS 72 M13B F cactgcttagagcgatgcACACTGCACTTTGAAATTGAACA Df MS 72 R gtTTCATTCCCCATACGTTACA											



Octanucleotide repeats

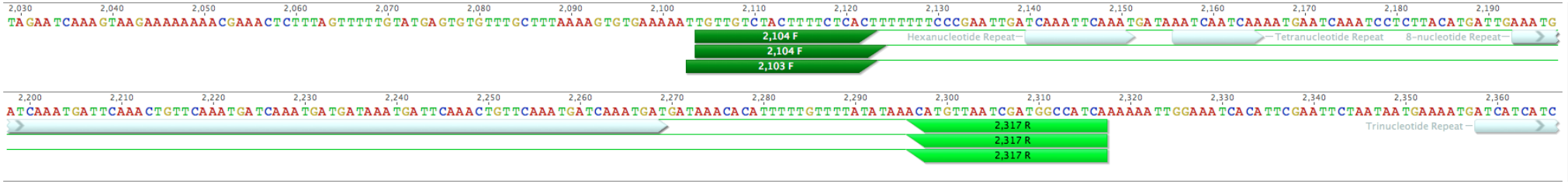
MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
54	ASGP 01 004 511	AAATGATC	AAATGATC	8	71	2,193-2,269	15	87	F: TTGTTGTCTACTTTTCTCACT R: TGATGGCCATCGATTAACATGT	52.7 58.2	215

Df MS 54 M13B F

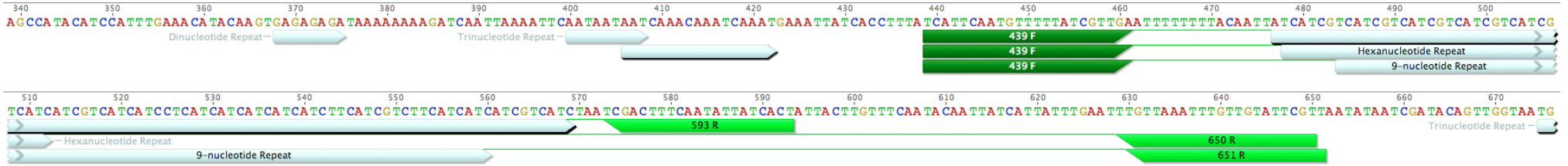
actgcttagagcgatgcTTGTTGTCTACTTTTCTCACT

Df MS 54 R

gttTGATGGCCATCGATTAACATGT



Nanonucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
25	ASGP 01 002 255	ACGATGATG	GTCATCATC	8	77	477-569	20	90	F: TCATTCAATGTTTTTATCGTTGA R1: AGTGATAATATTGAAAGTCGA R2: ACGAATACAACAAATTTAACAA	53.3 50.0 50.9	 155 212
Two different combinations of primers.											
Df MS 25 M13B F cactgcttagagcgatgcTCATTCAATGTTTTTATCGTTGA Df MS 25 R1 gtttAGTGATAATATTGAAAGTCGA Df MS 25 R2 gtttACGAATACAACAAATTTAACAA											



The trinucleotide repeat ATC is longer, normalised length 93 nt with 31 repeats and has 88.2 % perfection, the nanonucleotide repeat is shorter with a normalised length of 77 nt and 8 repeats, but it has a perfection of 89.6 %.

Nanonucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
29	ASGP 01 000 157	AACAACCAC	GGTTGTTGT	10	90	10,699-10,788	21	89	F: CCACCACAGTTCTTGTTCC R: CAGCATTGGCAGCGTTAGTG	56.1 60.2	325

Df MS 29 M13B F

cactgcttagagcgatgCCACCACAGTTCTTGTTCC

Df MS 29 R

tttCAGCATTGGCAGCGTTAGTG

Nanonucleotide repeats

MS #	Contig #	Rep. type	Reps.	Rep.	Nr1	Region nt	Score pts	Perfect %	Primers	Tm ˚C	Prod. bp
40	ASGP 01 001 769	AACAGCAGC	CAGCAACAG	9	83	918-1,000	26	90	F: GCAATCACAGCTTCCTGATCC R1: TGCCTTTGTATCTGATGCAGA R2: GAGCCATCCGAATTTTTGATCCT	59.3 57.4 59.4	 168 225

Two different combinations of primers.

Df MS 40 M13B F

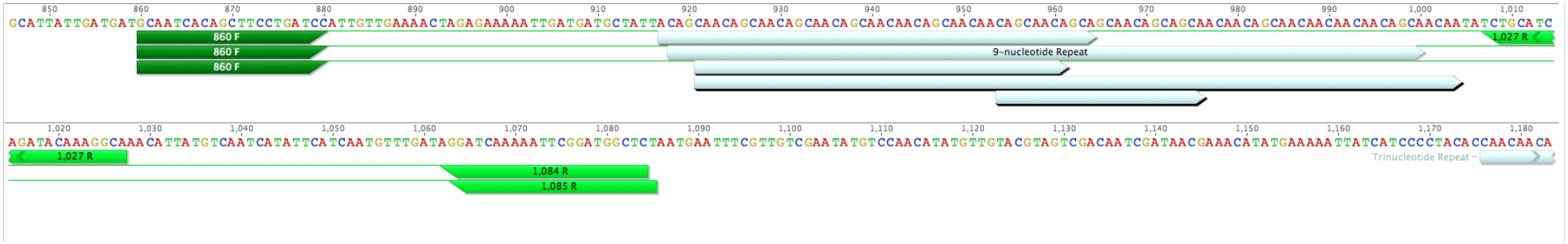
cactgcttagagcgatGCAATCACAGCTTCCTGATCC

Df MS 40 R1

ttTGCCTTTGTATCTGATGCAGA

Df MS 40 R2

gtttGAGCCATCCGAATTTTTGATCCT



Decanucleotide repeats											
MS #	**Contig #**	**Rep. type**	**Reps.**	**Rep.**	**Nr1**	**Region nt**	**Score pts**	**Perfect %**	**Primers**	**Tm ˚C**	**Prod. bp**
55	ASGP 01 010 607	AAAAAATAAC	TTTTTTGTTA	7	71	2,082-2,157	25	90	F: CGCACACACCCGAAACATTC R: ACAAAATCAAGTTCGACATCG	60.4 55.2	173
Df MS 55 M13B F cactgcttagagcgatgCGCACACACCCGAAACATTC Df MS 55 R gtttACAAAATCAAGTTCGACATCG											

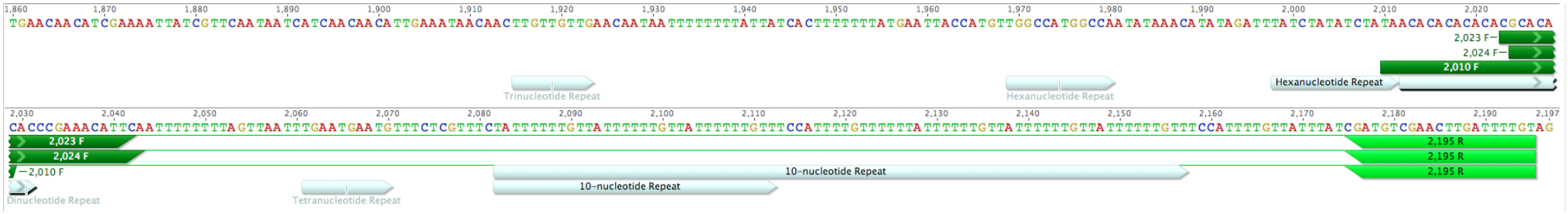


Table 6.3 – Detail characteristics of each microsatellite developed from the genome of *D. farinae* with an alignment of the microsatellite to the particular region of the genome segment. The higher the Phobos score of the microsatellite, the more likely it is predicted to perform well.

MS #: Unique name (number) of microsatellite, given based on total length of repeat sequence, the lower, the longer, irrespective of repeat type or length. Microsatellites are ordered by repeat class, i.e. dinucleotide, trinucleotide ... repeats # 1 is Df MS 1; **Contig #:** Name of original contig of the genome; **Repeat type:** standardised repeat sequence; a TC repeat is report as an AG type repeat; **Repeats:** number of full-length repeat units as n for longest repeat sequence

Nrl: normalised repeat length in nt; **Regionnucleotide**: position of longest repeat sequence; **ScorePhobos:** score for microsatellites in points; **Perfectpercentage**: perfection of repeats in longest repeat sequence; **Tm:** melting temperature of primer in ˚C according to primer3; and **Prod.:** length of PCR product in bp without tags and tails, for M13B and a half tail, add around 24 nt

Melting temperatures have been calculated following SantaLucia Jr (1998).

6.4 Discussion

One of the main aims of designing microsatellites is to optimise the information content of individual microsatellite markers or the allelic richness of the microsatellite, which lies in their degree of polymorphism. The degree of polymorphism is linked to the total length of the repeats or the number of repeat units in a microsatellite marker.

Microsatellites are historically short and perfect. Most microsatellites are limited to di- and trinucleotide repeats. They are short because of limitation in the traditional isolation methods and also of limitations associated with older sequencing machines to read the microsatellite bands. They are mainly perfect because imperfect microsatellite repeat areas are surrounded by nanosatellites, homopolymeric stretches, low complexity regions or/and semirepetitive regions known as cryptic simple sequences. Primers designed in these regions are likely to (partially) align in other similar sites as well, which will make the primers unspecific. Primer design software by itself cannot prevent primer design in low complexity regions. Normally microsatellites in regions of low complexity either failed or microsatellites in regions of low complexity have been eliminated; here, an attempt was made to design the primers outside the regions of low complexity. The allelic richness of imperfect microsatellites has rarely been analysed.

The proposed microsatellites show a lot of variation. Research questions could be formulated regarding their lengths, repeat units, composition, perfectness and the proportion of repetitive elements in the amplicon. They are long in total length as in maximum number of repeat units and they are long in terms of the length of the repeat unit itself. The microsatellites have been designed from genomic sequences, 11,600 contigs in 495 scaffolds. This allowed to maximise the total repeat length or the number of repeats of the proposed microsatellites in a way that is not possible with classical approaches to microsatellites. Microsatellites with these characteristics have rarely if ever been investigated. The microsatellites will not only offer a method to study and differentiate populations and, for example, estimate the size of these populations, but will also allow systematic analyses of microsatellite characteristics and genotyping success.

6.5 References

Arlian LG, Neal JS and Vyszenski-Moher DL (1999) Fluctuating hydrating and dehydrating relative humidities effects on the life cycle of *Dermatophagoides farinae* (Acari: Pyroglyphidae). *Journal of Medical Entomology* 36: 457-461.

Arlian LG and Morgan MS (2015) Reproductive biology of *Euroglyphus maynei* with comparisons to *Dermatophagoides farinae* and *D. pteronyssinus*. *Experimental and Applied Acarology* 66: 1-9.

Chan T-F, Ji K-M, Yim AK-Y, Liu X-Y, Zhou J-W, (Li R-Q, Yang KY, Li J, Li M, Law PT-W, Wu Y-L, Cai Z-L, Qin H, Bao Y, Leung RK-K, Ng PK-S, Zou J, Zhong X-J, Ran P-X, Zhong N-S, Liu Z-G and Tsui SK-W (2015) The draft genome, transcriptome, and microbiome of *Dermatophagoides farinae* reveal a broad spectrum of dust mite allergens. *Journal of Allergy and Clinical Immunology* 135: 539-548.

Colloff MJ (2009) *Dust Mites*. Springer, Dordrecht; pp 583.

Dusbábek F (1979) Dynamics and structure of mixed populations of *Dermatophagoides farinae* and *D. pteronyssinus*. In: Rodriguez JG (ed) *Recent Advances in Acarology*, vol 2. Academic Press, New York; pp 173-178.

Lee J, Kim JY, Yi M-h, Hwang Y, Lee I-Y, Nam S-H, Yong D and Yong T-S (2019) Comparative microbiome analysis of *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus,* and *Tyrophagus putrescentiae*. *Journal of Allergy and Clinical Immunology* 143: 1620-1623.

Mollet JA and Robinson WH (1996) Evaluating the dispersal of the american house dust mite (*Dermatophagoides farinae* Hughes (Pyroglyphidae)) using marked mites In: Mitchell R, Horn DJ, Needham GR and Welbourn WC (eds) *Acarology IX Proceedings*, vol 1. Ohio Biological Survey, Columbus, Ohio; pp 509-510.

Naegele A, Reboux G, Scherer E, Roussel S and Millon L (2013) Fungal food choices of *Dermatophagoides farinae* affect indoor fungi selection and dispersal. *International Journal of Environmental Health Research* 23: 91-95.

Peng J, Zhou Y, Jia H, Li L, Qian J, Han F, Yin H and Cui Y (2018) Transcriptomics-based identification of aquaporin diversity in the house dust mite *Dermatophagoides farinae* (Acariformes: Pyroglyphidae). *Journal of Insect Science* 18: e5.

SantaLucia Jr J (1998) A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. *Proceedings of the National Academy of Sciences of the United States of America* 95: 1460-1565.

Solarz K, Skubala P, Wauthy G and Szilman P (2016) Body size variability in different forms of heteromorphic males in populations of the house dust mite *Dermatophagoides farinae* Hughes 1961 (Acari: Astigmata: Pyroglyphidae). *Annales Zoologici (Warsaw)* 66: 329-336.

Teplitsky V, Mumcouglu KY, Dalal I, Somekh E and Tanay A (2004) House dust mites on the skin and clothes of atopic dermatitis patients. *Journal of Allergy and Clinical Immunology* 113: S296.

Thet-Em T, Tungtrongchitr A, Tiewcharoen S and Malainual N (2012) Multiplex PCR for identifying common dust mites species (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis*). *Asian Pacific Journal of Allergy and Immunology* 30: 224-230.

van Asselt L, Wauthy G and Grootaert P (1996) Spatial distributions of *Dermatophagoides farinae* Hughes and *Dermatophagoides pteronyssinus* (Trouessart) (Pyroglyphidae). In: Mitchell R, Horn DJ, Needham GR and Welbourn WC (eds) *Acarology IX Proceedings*, vol 1. Ohio Biological Survey, Columbus, Ohio; pp 71-74.

Wang Y-M, Liu X-Y, Jiang C-L, Huang L-N, Sun X and Liu Z-G (2013) [Ultrastructure of the digestive system in *Dermatophagoides farinae* (Acariformes: Pyroglyphidae)]. *Chinese Journal of Parasitology and Parasitic Diseases* 31: 490-492.

Wang Y-M, Wu L, Wu Y-Y, Li M, Yang L-T, Huang L-N, Sun X and Liu Z-G (2014) Ultrastructure of the integument and haemocoele in *Dermatophagoides farinae* (Hughes) (Acariformes Pyroglyphidae). *Chinese Journal of Zoology* 30: 23-26.

Yang R, Niu D, Zhao YE, Gong X-J, Hu L and Ai L (2019) Function of heat shock protein 70 in the thermal stress response of *Dermatophagoides farinae* and establishment of an RNA interference method. *Gene* 705: 82-89.

Yue-Ming W, Xiao-Yu L, Li-Nian H, Xin S and Zhi-Gang L (2013) Ultrastructure of the reproductive system in *Dermatophagoides farinae* (Acariformes Pyroglyphidae) observed with transmission electron microscopy. *Acta Entomologica Sinica* 56: 960-964.

7 Cross species conservation of microsatellites of *D. farinae* microsatellites in the genome of *D. pteronyssinus*

7.1 Introduction

Dermatophagoides farinae and *D. pteronyssinus* are known as closely related species in the house dust mite family Pyroglyphidae (Acariformes). *D. farinae* is vernacularly known as the American house dust mite species, and *D. pteronyssinus* as the European house dust mite species, and often considered sister species. In the last thirty to fifty years, both species have been extensively admixed by humans. Nevertheless, there are still new geographical records for *Dermatophagoides* species (Martinez Canzonieri et al., 1995).

Recently in the United States, some 7,000 bedrooms have been studied for the ratio of *D. pteronyssinus and D. farinae*. Multivariable regression for several, forensically interesting features resulted in different odds ratios. The ratio of *D. pteronyssinus and D. farinae* can be pre*dictors for* age, 1-17 versus 18+ years, for race/ethnicity, non-Hispanic black versus others, versus Mexican Americans, versus non-Hispanic white, for level of urbanization, for type of home (multifamily, single family, mobile home), for number of people in a household, for presence of dog(s) or cockroaches or mildew or children, for floor covering, for room humidity and for room temperature. *D. pteronyssinus and D. farinae* were only informative for large scale regional differences such as the US census regions West versus Midwest; *D. pteronyssinus and D. farinae* were not informative for gender, residence time, presence of cat(s) and presence of mattress cover (Salo et al. 2018). If just by looking at the ratio of *D. pteronyssinus and D. farinae,* forensically valuable information can be obtained, how much could be gained by high-resolution of genotyping these two species with microsatellites?

Molecularly, *D. microceras* is currently the sibling species to *D. farinae*, and *D. evansi* is currently the sibling species to *D. pteronyssinus*, Figure 7.1. *D. evansi* has first been found in feather pillows; *D. microcercas* has been reported from house dust, mattresses, and feather cushions (Hughes, 1976).

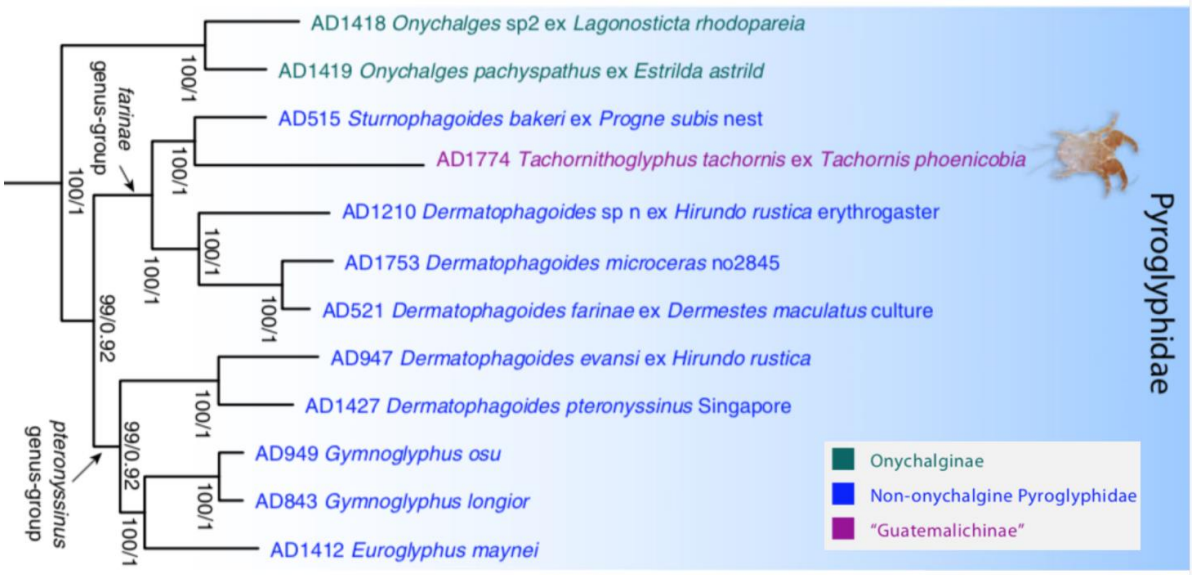


Figure 7.1 – Molecular phylogentic tree showing the relationship of *Dermatophagoides farinae* and *D. pteronyssinus* (Klimov et al., 2016). Maximum likelihood analysis applied to six sequence fragments rDNA stems, rDNA loops, EF1-a, SRP54, HSP70, and CO1. For each node, bootstrap support values and internode certainty indices are given.

Molecular trees are limited by the availability of sequences. For most mite species, molecular characterization is still illusive. Even in such a well-known genus as *Dermatophagoides*, the actual number of described species is uncertain. Table 7.1 lists some of the better known *Dermatophgoides* species that still await molecular characterization.

***Dermatophagoides* species**
D. africanus Hughes, 1954
D. chirovi Adieva, 1991
D. culinae De Leon, 1963
D. crassus (Can.)
D. deanei Galvao and Guitton, 1986
D. delarnaesis Sellnick, 1958
D. longior (Trouessart, 1897)
D. maynei (Cooreman, 1950)
D. neotropicalis Fain, and van Brunswijk, 1973
D. saitoi (Sasa)
D. scheremetewskyi Bogdanov, 1864
D. sclerovestibulatus Fain, 1975
D. siboney Dusbabek, Cuervo, and Delacruz, 1982
D. sorensoni Tibbetts
D. takeuchii (Sasa)
D. toxopei (Oudemans, 1928)

Table 7.1 – Any or all of the *Dermatophagoides* species in this list could phylogenetically reside between *D. farinae* and *D. pteronyssinus*, highlighting how relative the concept of sister-species are especially in acarology.

Some of these *Dermatophagoides* species have interesting features. For example, *D. toxopei* is a species that has been collected from the silk web of another mite species, *Schizotetranychus asparagi*, on asparagus plants (De Leon, 1963). *D. culinae* has been discovered in a kitchen flour bin with self-rising biscuit flour in Tennessee, USA (De Leon, 1963). It is not known whether this species feeds directly on baking powder in the flour or whether a mite product chemically interacts with the baking powder and inactivates it, but the species has become known as the one that prevents biscuit from raising. *D. scheremetewskyi* is a free-living stored product mite

that is transitioning to a parasitic mite, which can cause dermatitis in humans (Sassa and Shingai, 1958). *D. sorensoni* is nasal mite, which has been extracted from the nasal passages of a Korean woodpecker (Tibetts, 1955).

7.2 Material and methods

The microsatellites of *D. farinae* described in the foregoing chapter where compared with genome of *D. pteronyssinus.* Sequences were analysed with the help of the Geneious platform, version R10, from Biomatters. All individual microsatellites of *D. farinae* were blasted against the genome of *D. pteronyssinus.* The Basic Local Alignment Search Tool, BLAST+, version 2.8.0, was downloaded from the NCBI website and run from within Geneious R10 (Camacho et al., 2009). The draft genome and transcriptome of *D. farina* has been reported by Chan et al. (2015). Two years later, the first draft genome for *D. pteronyssinus* has been announced (Waldron et al., 2017), which has been used here. Recently, a higher quality genome and transcriptome of *D. pteronyssinus* has been published (Liu et al., 2018).

7.3 Results

The cross-species conservation of microsatellites ranges between 50 % for octanucleotide repeats and 100 % for heptanucleotide, nanonucleotide, and decanucleotide repeats, albeit covering only very small numbers. The two largest categories, dinucleotide repeats exhibited 96 % (50/52) conservation, and trinucleotide repeats were conserved for 77 % (21/27) of the microsatellites. The overall conservation of *D. farinae* microsatellites in the genome of *D. pteronyssinus* is 86 % (95/104), Table 7.2.

Presence and absence of individual microsatellites of *D. farinae* in the genome of *D. pteronyssinus*

Dinucleotide repeats	n	MS id #
D. farinae	52	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13 14, 17, 18, 19, 25, 29, 32, 34, 35, 39, 40, 41, 43, 44, 46, 51, 54, 55, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 94, 96, 97, 98, 99, 100, 101
D. pteronyssinus	50	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13 14, 17, 19, 25, 29, 32, 34, 35, 39, 40, 41, 43, 44, 46, 51, 54, 55, 77, 78, 79, 80, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 94, 96, 97, 98, 99, 100, 101
Trinucleotide repeats		
D. farinae	27	4, 8, 15, 16, 20, 21, 22, 23, 24, 26, 27, 28, 30, 31a, 31b, 33, 36, 37, 38, 42, 45a, 45b, 47, 48, 49, 50, 78
D. pteronyssinus	21	4, 8, 16, 20, 21, 23, 24, 26, 27, 28, 30, 33, 36, 37, 38, 45a, 45b, 47, 48, 50, 78
Tetranucleotide repeats		
D. farinae	8	52, 59, 60, 64, 66, 67, 71, 73
D. pteronyssinus	6	52, 59, 60, 64, 71, 73
Pentanucleotide repeats		
D. farinae	4	58, 69, 75, 76
D. pteronyssinus	3	58, 69, 75
Hexanucleotide repeats		
D. farinae	11	53, 56, 57, 61, 62, 63, 65, 68, 74, 93, 95
D. pteronyssinus	9	56, 57, 61, 62, 65, 68, 74, 95
Heptanucleotide repeats		
D. farinae	2	70, 72
D. pteronyssinus	2	70, 72

Octanucleotide repeats		
D. farinae	1	54
D. pteronyssinus	0	-
Nanonucleotide repeats		
D. farinae	3	25, 29, 40
D. pteronyssinus	3	25, 29, 40
Decanucleotide repeats		
D. farinae	1	55
D. pteronyssinus	1	55

Table 7.2 – Cross-species conservation of microsatellites in *Dermatophagoides* species. Listed are the number n of microsatellites per repeat category for each of the two species and the microsatellite id number, MS id # of each microsatellite, based on the list in the foregoing Chapter. Only one microsatellite with octancleotide repeats have been identified in *D. farinae*, this microsatellite is absent from the genome of

7.4 Discussion

This percentage of cross-species conservation of microsatellites is quite high for sister species in mites, Table 7.3. *Dermatophagoides* species behave in a similar matter as *Colomerus* and *Tetranychus* species. Even at the genus level, the conservation does not seem to ebb off much. So far, studies at the family level have not yet been conducted for mites.

This shows that for closely related species microsatellites can be borrowed from neighbouring species. Despite this high rate of conservation of microsatellites in mites, Ge et al. (2013) advices not to make use of it, if at all possible. A high rate of null alleles and low or very low allelic diversity, and the very labour-intensive process of improving transferred microsatellite primers might be counter-productive. Ge et al. (2013) recommends instead developing microsatellites from scratch for every new species.

From a forensic point of view, this means that very few species-specific microsatellites will be available for the analysis of dust mite consortia in a metagenomic kind of approach, which would be desirable for routine work, taking advantage of molecular biology technicians. Without the use of species-specific primers, species identification of individual mites will be necessary in forensic cases, limiting the approach to the realm of specialised acarologists.

Cross-species application of microsatellites in Acari

Original species	Transfer species	Region	Success	Reference
Dermatophagoides farina	*D. pteronyssinus*	UK	95/104	This work
Colomerus vitis	*Calepitrimerus C. vitis*	Australia	3/4	Carew et al., 2004
Tetranychus urticae	*T. truncatus*	China	36/205	Ge et al., 2013
	T. turkestani	France	5	Bailly et al., 2004
	T. cinnabarinus	China	3/19	Li et al., 2009
	T. turkestani	Spain	10/11	Sabater-Muñoz et al., 2012
	T. evansi	Spain	8/11	
	T. okinawanus	USA	8/11	
	Eotetranychus			
	E. orientalis	Spain	8/11	
	E. banksi	Spain	9/11	
	Panonychus citri	Spain	8/11	
	Oligonychus			
	O. perseae	Spain	8/11	
	Aplonobia			
	A. histricina	Spain	7/11	
	Typhlodromus			
	T. phialatus	Spain	8/11	
	Neoseiulus			
	N. californicus	Comm.	7/11	
	N. barkeri	Spain	8/11	
	Euseius stipulatus	Spain	7/11	
	Phytoseiulus			
	P. persimilis	Comm.	7/11	
	Amblyseiulus			
	A. swirski	Comm.	6/11	
	A. andersoni	Comm.	9/11	
	A. cucumeris	Comm.	6/11	
T. truncatus	*T. piercei*	China	22/36	Ge et al., 2013
	T. ludeni	China	2/36	
	T. phaselus	China	4/36	

Table 7.3 – Success rate of cross-species application of microsatellites
Success: Microsatellites successful in transferred species/microsatellites in original species; **Comm.:** Commercial source, origin uncertain.

7.5 Conclusions

Around 80 % of microsatellites are conserved in sister species and possibly also in sister genera. Careful analyses suggest that this might be more of a problem than an easy shortcut.

7.6 References

Bailly X, Migeon A and Navajas M (2004) Analysis of microsatellite variation in the spider mite pest *Tetranychus turkestani* (Acari: Tetranychidae) reveals population genetic structure and raises questions about related ecological factors. *Biological Journal of the Linnean Society* 82: 69–78.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K and Madden TL (2009) BLAST+: architecture and applications. *BMC Bioinformatics* 10: e421.

Carew ME, Goodisman MAD and Hoffman AA (2004) Species status and population genetic structure of grapevine eriophyoid mites. *Entomologia Experimentalis et Applicata* 111: 87-96.

Chan T-F, Ji K-M, Yim AK-Y, Liu X-Y, Zhou J-W, (Li R-Q, Yang KY, Li J, Li M, Law PT-W, Wu Y-L, Cai Z-L, Qin H, Bao Y, Leung RK-K, Ng PK-S, Zou J, Zhong X-J, Ran P-X, Zhong N-S, Liu Z-G and Tsui SK-W (2015) The draft genome, transcriptome, and microbiome of *Dermatophagoides farinae* reveal a broad spectrum of dust mite allergens. *Journal of Allergy and Clinical Immunology* 135: 539-548.

De Leon D (1963) A new Dermatophagoides: it prevents the rising of self-rising flour (Acarina: Epidermoptidae). *Florida Entomologist* 63: 247-250.

Ge C, Sun JT, Cui YN and Hong XY (2013) Rapid development of 36 polymorphic microsatellite markers for *Tetranychus truncatus* by transferring from *Tetranychus urticae*. *Experimental and Applied Acarology* 61: 195–212.

Hughes AM (1976) *The Mites of Stored Food and Houses. 2nd edition*. Her Majesty's Stationary Office, London; pp 400.

Klimov PB, Bochkov AV and OConnor BM (2016) Phylogenetic position of the house dust mite subfamily Guatemalichinae (Acariformes: Pyroglyphidae) based on integrated molecular and morphological analyses and different measures of support. *Cladistics* 32: 261-275.

Li T, Chen X-L and Hong X-Y (2009) Population genetic structure of *Tetranychus urticae* and its sibling species *Tetranychus cinnabaribus* (Acari: Tetranychidae) in China as inferred from microsatellite data. *Annals of the Entomological Society of America* 102: 674-683.

Liu X-Y, Yang KY, Wang M-Q, Kwok JS-L, Zeng X, Yang Z, Xiao X-J, Lau CP-Y, Li Y, Huang Z-M, Ba J-G, Yim AK-Y, Ouyang C-Y, Ngai S-M, Chan T-F, Leung EL-H, Liu L, Liu Z-G and Tsui SK-W (2018) High-quality assembly of *Dermatophagoides pteronyssinus* genome and transcriptome reveals a wide range of novel allergens. *Journal of Allergy and Clinical Immunology* 141: 2268-2271.

Martinez Canzonieri CE, De Grosso ML, Baggio D and Crocce J (1995) First record of the house dust mite *Dermatophagoides pteronyssinus* in Tucuman (Argentinian Republic). *Acta Zoologica Lilloana* 43: 229.

Sabater-Muñoz B, Pascual-Ruiz S, Gómez-Martínez MA, Jacas JA and Hurtado MA (2012) Isolation and characterization of polymorphic microsatellite markers in *Tetranychus urticae* and cross amplification in other Tetranychidae and Phytoseiidae species of economical importance. *Experimental and Applied Acarology* 57: 37-51.

Sassa M and Shingai H (1958) Occurrence of the mite *Dermatophagoides scheremetewskyi* Bogdanoff, free-living in albumine tannate stored in dispensaries. *Japanese Journal of Experimental Medicine* 28: 1-10.

Tibetts T (1955) A new nasal mite from a Korean woodpecker. *Proceedings of the Entomological Society of Washington* 57: 197-201.

Waldron R, McGowan J, Gordon N, McCarthy C, Mitchell EB, Doyle S and Fitzpatrick DA (2017) Draft genome sequence of *Dermatophagoides pteronyssinus*, the European house dust mite. *Genome Announcements* 5: e32.

8 Research journals covering biological aspects of forensic sciences and legal medicine

8.1 Introduction

The number of predatory journals in forensic sciences is steadily increasing. Data published in such journals, for example the base temperature of an insect species involved in a post-mortem interval (PMI) estimation, has not been peer reviewed, and, if used in a court case, can lead to miscarriages of justice. It is therefore of the outmost importance that future practitioners be able to differentiate between reliable sources of forensic data that conform to the Daubert Standard and non-reliable sources that do not meet the criteria for validity of scientific evidence (Daubert and Merrell Dow Pharmaceuticals, 1993). It is also of great importance to prevent future publication in predatory journals. For example, members of both the European Association of Forensic Entomology (EAFE) and the North American Forensic Entomology Association (NAFEA) not only publish in predatory journals, they have become editors of these journals as well, giving these predatory journals a false appearance of credibility or legitimacy. This clearly shows that either a black list of predatory journals in forensics or a white list of non-predatory journals is disparately needed. While a black list would be highly entertaining, it would equally be controversial and very soon out of date. A white list of establisher and widely recognized forensic journals with a rigorous peer-review system on the other hand would be less controversial and very useful for a much longer period of time. Since 2015, a small group of forensic scientists from the UK, Germany, Spain, and the USA, that closely monitors closely predatory journals in forensics, has come up with a white list of current research journals covering biological aspects of forensic sciences and legal medicine.

8.2 What are predatory journals

8.2.1 Who pays

Traditional science journals are subscription-based. Either individuals or libraries subscribe to a journal by paying a yearly subscription fee. Some journals come as part of the membership fees of a learned or professional society. In the end, it is the reader who pays for at least for part of the journal. In many cases, authors also have to pay for part of the production costs of their paper. There are two guardians for

the quality of the journal, meaning the quality of the articles in the journal; one, the publisher through the editor of the journal as a gate keeper using peer review of the submitted manuscripts to decide which articles to publish, and, two, the reader, who might decide directly, or indirectly through a library committee, to end a subscription if the quality slips. This means that the journal or publisher is ultimately under the final control of the reader.

This all changed formerly in 2002 and 2003 with the Budapest Open Access (OA) Initiative, the Bethesda Statement on OA Publishing, and the Berlin Declaration on OA to Knowledge in the Sciences and Humanities although the first OA journals had already been in existence for a few years. One of the first OA journal in the natural sciences was *Psycoloquy* (1989-2002) sponsored by the American Psychological Association followed by *Solstice: An Electronic Journal of Geography and Mathematics* (1990-present) published by the Institute of Mathematical Geography at the University of Michigan, USA, interestingly followed by several law journals like the *eLaw Journal* (1993-present) currently published by the School of Law, Murdoch University, Australia, and *Journal of Criminal Justice and Popular Culture* (1993-present), published by the School of Criminal Justice, University at Albany, USA. Already in 2000, the first forensic OA journal saw the light of day, *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* published by A. K. Aggrawal, a professor of forensic medicine at the Maulana Azad Medical College in New Delhi, India. In OA journals, the authors now pay for everything and the reader no longer pays anything. There are some cases where OA journals are partially or entirely subsidized by academic or governmental institutions. An example is the journal *Acarologia* supported by the French government. In OA, the control of the reader over the publisher disappeared. This opened the publication system up to predation by predatory publishers who prey on authors or the ego of authors willing to pay for publishing without rigorous peer review. Beall (2012) coined the term predatory publisher in 2010. Knoll (2014) and Kannan (2015) call predatory journals parasites of OA. This might suggest that OA is not such a good thing. Far from it. OA is a huge step forward for science, society, and forensics. So much so that, for example, the national research funding bodies in eleven countries

in Europe announced Plan S to replace the traditional subscription-based publishing system completely with OA, including forensics (Else, 2018). Two of the World's largest private biomedical funders, The Welcome Trust in the UK and the Bill and Melinda Gates Foundation in the USA have just announced their intent to implement this plan.

Many of the subscription-based journals have already become hybrid journals, where the authors can choose by paying an (increased) fee to have their article OA while the rest of the articles remain locked for non-paying readers.

8.2.2 Peer review

Scholarly peer review and the rigor of peer review have been the single most important tool in maintaining quality of scientific publications. Peer-reviewed publications are a requirement of the profession. For example, the American Academy of Forensic Sciences (AAFS) criteria for membership require the applicant to have made some significant contribution to the literature of forensic science; the American Board of Forensic Entomology (ABFE) criteria for admission as a member requires to possess a minimum of one peer reviewed publication on a subject germane to the field of forensic entomology (senior authorship is not required). Peer review is also the fundamental principle underpinning forensic evidence as expressed in the Daubert Standard (Daubert and Merrell Dow Pharmaceuticals, 1993; Norko, 2014; Shoucair, 2018).

There have been several high-profile demonstrations of the lack of effective peer review in predator journals.

The first demonstration that some scientific conference lack functional peer review started in the field of computer science. Three PhD students from the Massachusetts Institute of Technology created a freely available programme called SCIgen that can generated nonsensical research papers in computer science (Bohannon, 2015b). People could anonymously use the software to make their papers for submission to conference proceedings. In 2013, 122 of these papers were retracted by publishers (Labbe and Labbe, 2013).

To demonstrate the lack of functional peer review for journal manuscripts needed some more work. This proof came for the life sciences when Bohannon (2013) submitted under pseudonyms and with made-up affiliations 304 fatally flawed manuscripts about substances from lichens against cancer cells to OA journals. During the experiment 29 journals went under and another 20 did not finish the submission process. In this sting operation, 157 bogus manuscripts got accepted by 157 journals, 93 out of 111(84 %) of these journals were on Beall's black list of potential, possible or probable predatory journals and 73 out of 158 (46 %) were on DOAJ's whitelist. DOJA contained 16 journals that were on Beall's list. This showed that 84 % on Beall's list did not have a functional peer review system and were indeed predatory and 16 % were not. But it also showed that the whitelist had 46 % of journals that were predatory.

8.2.3 Spurious editors and authors

Masic (2017) describes how he became the editor in chief of the *Journal of Forensic Anthropology*, published by OMICS International Group and his experience with the publisher. The publisher has now shut down the journal and removed any traces of it from the web.

Hoss Cartwright, a fictional character from the Western series Bonanza became editor of the *International Journal of Agricultural Innovations and Research* and four other journals, Peter Uhnemann, a name borrowed from the German satirical magazine Titanic, became editor of *Molecular Biology*, and Borat Sagdiyev, a satirical fictional character created by comedian Sacha Baron Cohen became an editor of *Immunology and Vaccines* in sting experiments by Burkhard Morgenstern, a bioinformatics professor from the University of Göttingen (Marcus and Oransky, 2016).

Anna O. Szust, in Polish oszust means 'fraud', is a fictional scientist without any real publication record in a sting experiment. Forty out of 120 journals on Beall's black list accepted Szust as editor, 4 instantly appointed her as editor in chief, 8 journals out of 120 of DOAJ's whitelist appointed the fake editor, and none out of 120 journals of Web of Science accepted Szust application (Sorokowski et al., 2017).

Spurious authors go back a long time. For example, in 1978, Galadriel Mirkwood, the name of an Afghan hound, featured as a fictional co-author on a paper in the *Journal of Experimental Medicine*; in 1987, a fictional Stronzo Bestiale, in Italian 'giant asshole', became co-author on two papers that are still listed in Web of Science, one with 136 citations and the other with 27 (Marcus and Oransky, 2016). Nonsensical manuscripts authored by Margaret Simpson, Kim Jong Fun, and Edna Krabappel have been accepted by two journals. A proceedings paper on the development of wind generators from 2017 authored by Bart Simpson, Homer Simpson, Xuesong Zhou and others with the first two authors coming from the University of Springfield, Department of Nuclear Power Engineering in Springfield, Illinois, can be found on Web of Science.

8.3 Predatory journals and forensics

The potential danger of predatory journals to forensics was first voiced by Knoll (2014), arguing that research findings must be reliable enough to be presented as testimony in court. The central precept in forensics is that research findings and data must be sufficiently reliable to be proffered as testimony or evidence in court. It can also be formulated as a question: How do courts protect themselves against predatory research findings and data? As for data, using the traditional route of publishing might take the better part of a year; in a predatory journal this might get reduced to a few days and would be easily possible during the proceedings of a court case.

The first editor of a forensic journal to warn against the perils of predatory publishing was Peternelj-Taylor (2015) for forensic nursing. Byard (2016) is concerned that may not only be used to legitimize fringe theories but to validate bogus experts in forensics as well.

The danger of hijacked journals for the whole of science has been reviewed by Dadkhah et al. (2017a). Noga-Styron et al. (2017) describe the experience as authors in predatory journals and the knowledge of predatory publishing of members of the Academy of Criminal Justice Sciences and the American Society of Criminology.

One might think that predatory journals are a fringe phenomenon, responsible for only a small fraction of the journals in a field. Far from it. In emergency medicine, for example, about half of the open access journals might already be predatory (Hansoti et al., 2016). Forensic sciences are heading in the same direction.

Forensic articles are not limited to predatory journals in forensic sciences. For example, an article about diatom extraction in the diagnosis of drowning appeared in a journal on clinical and experimental pharmacology.

8.4 Recognizing predatory journals

On the surface, that seems easy: if it looks too good to be true, it probably is (Butler, 2013b). In a more systematic approach, it becomes much more difficult.

There are some giveaways to spot a predatory journal. Most of these indicators go back to recommendations given by Beall on his former website.

- Name. The journal name or logo resembles other well-known journals, it might well be a hijacked journal, a predatory journal looking like a well-established journal (Butler, 2013a; Bohannon, 2015a; Gasparyan et al., 2015; Jalalian and Dadkhah, 2015; Dadkhah et al., 2016; Dadkhah et al., 2017b).
- E-mail. Publisher or editor have non-company or non-institutional email addresses like gmail, yahoo, and so on (Stojanovski and Marušić, 2017).
- ISSN. If the journal does not have an eight-digit International Standard Serial Number (ISSN), it is certainly predatory. The ISSN is only issued to uniquely identify a serial publication, it does not involve any quality evaluation. Many predatory journals do have an ISSN to look legitimate, but some predatory publishers do not even care to ask for one. Since 2014, the ISSN Centre can refuse issuing ISSNs to publishers that provide misleading information. ISSN is now developing with support of UNESCO a service called ROAD, the Directory of Open Access scholarly Resources (Oury, 2017).

- Language. The website of the journal is in poor English, rich in typos, and dead links (Kearney and The INANE Predatory Publishing Practices Collaborative, 2015; Dewan and Shah, 2016).
- Origin. The geographic association of the journal's name is misleading. For example, is a *British Journal of* or an *American Journal of* indeed rooted in that particular country? Beall (2013) noted that there might be more *British Journals of* located in Pakistan than in the United Kingdom itself.
- Field. The scope of the journal should not be bizarrely broad; however, see mega journals like PLOS ONE or Scientific Reports.
- Costs. The fee or costs to get published, often Article Processing Charges (APC) called, can range widely between as low as $ 20 and as high as $ 5,000 (Kearney and The INANE Predatory Publishing Practices Collaborative, 2015; Xia, 2015). At the lower end of charges, predatory journals are easy to recognize. Some of the most streamlined OA mega journals such as *PLOS ONE*, published by the non-profit publisher Public Library of Science or Scientific Reports published by the for-profit Nature Publishing Group charge in 2018 around $ 1,595 or $ 1,760, respectively. Unfortunately, the average fee of predatory journals targeting forensic scientist is around $ 1,079 (Byard, 2016), not too far off from a mainstream journal. Many predatory journals do not advertise their fees at all and the costs are only disclosed after submitting or displaying the manuscript on their website (Beall, 2012). The average APC for an article across all predatory journals is only around $ 180 (Shen and Björk, 2015).
- Editors. Most, if not all, members of the editorial board should look familiar. If only one or two are, it is very bad sign, worth if some editorial members are listed twice, some only by their first name, or, for example, in a predatory forensic journal, editorial members of the National University of Cordoba, the Lebanese American University, and Northumbria University are all situated in the USA. Editorial members may be listed against their will (Butler, 2013a; Nicoll and Chinn, 2015). Some predatory publishers fraudulently list

established researches as staff reviewers or contributors (Dadkhah et al., 2016; Gasparyan et al., 2016). Spears discovered that in 2015, the *Journal of Spectroscopy and Molecular Physics* stole the picture of Sir William Richard Doll, who was a pioneer in lung cancer epidemiology and died in 2005, and named him Professor Richard Turner.

- Editor in Chief. Many predatory journals in forensics do have an editorial board but do not have an editor in chief, whereas all non-predatory journals in forensics do have an editor in chief.
- Speed. The offer of an unrealistic fast peer review.
- Canvassing. Many predatory journals bombard potential authors with e-mails to solicit manuscripts or invite to become editorial members, in short, journal phishing. If the journal has appeared in your e-mail inbox unsolicited, it is likely predatory (Clemons et al., 2017; Dadkhah et al., 2017a). In an analysis of 300 e-mail invitations over a period of a year, 79 % originated from publishers on Beall's list (Moher and Srivastava, 2015). Most invitations came from the OMICS Publishing Group (69), SciDoc Publishers (21), Jacobs Publishing (13), MedCrave (10), the Center for Promoting Ideas (9), Aperito Online Publishing (7) and Austin Publishing Group (7).
- Submission. If the journal asks to submit the manuscript by e-mail instead of online, it is probably not professional (Shamseer et al., 2017).
- Impact Factor. Most subscription-based journals are being indexed which means covered by literature retrieval services or bibliographic databases such as Web of Science, formerly Web of Knowledge, originally run by the Institute for Scientific Information (ISI), now maintained by Clarivate Analytics, formerly Thomson Reuters, Scopus produced by Elsevier, Ovid provided by Wolters Kluwer, MEDLINE (Medical Literature Analysis and Retrieval System Online) compiled by the United States National Library of Medicine (NLM), CAB Direct advanced by the Centre for Agriculture and Bioscience International or EBSCO Information Services, which provides Criminal Justice Abstracts. The Institute for Scientific Information was the first to provide

widespread citation indexing by recording the number of citations articles in a particular journal acquired during the first two years after publishing, divided by the number of publications during the same period, resulting in the now famous impact factor, which is published in the annual Journal Citation Reports. Journals with an impact factor have been considered established. To achieve this recognition instantly, predatory journals started to report impact factors not obtained through one of the established citation indexing services but derived from equally predatory companies, spurious alternative impact factors or, in short, fake impact factors (Gutierrez et al., 2015; Xia and Smith, 2018). A new journal with a recently acquired impact factor might still be predatory; the selection to add a journal to, for example, Web of Science or Scopus, is not perfect. Many predatory journals lie about their indexing (Kebede et al., 2017).

- Affiliation. The association of a journal with a society or a professional organisation is the easiest to recognize and the most reliable feature to indicate that a less-known journal is most likely not predatory. This does not take away that some of the best-established journals have no affiliation, for example, *Nature*, *Forensic Science International*, or *Forensic Science, Medicine and Pathology*.
- Guest editor. A more difficult to spot trap occurs if a predatory publisher succeeds to recruit a known scientist as a guest or honorary editor (Johal et al., 2017).

Recognizing better disguised predatory journals becomes more challenging and much more time consuming, to a point, where it is no longer practical for an average author who wants to choose a journal for a manuscript to make the distinction. Beall proposed that authors should acquire the skill of scholarly publishing literacy in respect to predatory journals, the ever increasing numbers of predatory journals and articles in these journals suggests that this is still a long time out (Beall, 2013).

8.5 Lists

8.5.1 Black lists of publishers

The easiest and fasted solution for identifying a potential, possible, or probable predatory journal is to look it up on a black list. Such a black list including a blog has been maintained for five years by the librarian of the Auraria Library of the University of Colorado at Denver, USA, Jeffrey Beall, and became well known as Beall's list. It was a list of potential, possible or probable predatory scholarly open-access publishers. The Scholarly Open Access website started out in 2011 with only 18 publishers and reached in 2016 923 potential, possible or probable predatory publishers. After Beall removed his lists from the web, several people put their archived versions of Beall's list on the web. The organisation web.archive.org also hosts archived versions.

There is now a 'List of Predatory Publishers' by Stop Predatory Journals. This list was built by an independent group which wishes to remain anonymous in order to avoid the harassment suffered by Beall.

The biggest problem with black-listing publishers is that it makes it very difficult for big publishers to change. A publisher with more than a hundred journals will struggle to raise the standards of all its journals at the time. How many bad apples (journals) would be acceptable in a portfolio to leave a black list of publishers?

The threat of law suits makes black lists quite challenging (Beall, 2012; New, 2013; Beall, 2017).

8.5.2 Black lists of journals

In addition to his list of potential, possible or probable predatory scholarly open-access publishers, Beall maintained two more lists, a list of potential, possible or probable predatory scholarly open-access standalone journals and a list of hijacked journals. In 2013, there were 126 questionable standalone journals, in 2016, that number rose to 882 journals. Stop Predatory Journals offers also a 'List of Predatory Publishers'; it only contains one single possibly predatory forensic journal, the

Journal of Research in Forensic Medicine and Toxicology and five law journals. In addition, Stop Predatory Journals has an alphabetical 'List of Hijacked Journals'.

There is (not yet) a public black list of predatory journals brought out by publishers. The reason for this is the size of such a list. Some OA companies publish a large number of journals, one of the largest, OMICS International and its subsidiaries publishes 700 clinical, medical, life sciences, and engineering and technology journals and hosts 3,000 scholarly conferences per year. Making a very conservative estimate of only 11 journals per publisher , this would translate to ~ 11,000 potential, possible or probable predatory scholarly open-access journals for 2016 (Shen and Björk, 2015). It is estimated that in 2014 alone, 420,000 articles were published in predatory journals.

A scholarly analytics company from Beaumont, Texas, Cabell's offers for fee-paying customers a blacklist. As of 2018, Cabell's has 10,410 journals for all disciplines in its blacklist.

An actual list of predatory journals and their publishers exists for the flied of dermatology (Maddy and Tosti, 2017). The authors extracted and evaluated 76 predatory journals from Beall's list of publishers and published it in the *British Journal of Dermatology*. Later, these journals were subjected to a weighted ranking system based on predatory criteria. Of the 76 dermatology journals, 68 were identified as predatory journals, 8 as journals with predatory practices (Tosti and Maddy, 2017).

On a website of Uppsala University (The Ethics Blog), two faculty members maintain since two years a black list of journals in bioethics (Where to publish and not to publish in bioethics – the 2018 list).

8.5.3 White lists of publishers

The Open Access Scholarly Publishers Association (OASPA), a trade association representing the interests of OA journal publishers, maintains a rather short list of its members on the web, among which are several University Presses like Cambridge, Oxford or Stockholm, some of the major publishing companies like Public Library of Science (PLOS), Springer Nature, Taylor & Francis, or Wiley and Sons, but also

individual journals like *eLife*, *PerrJ*, or the *Journal of Digital Forensics, Security and Law*. To become a member, the publisher has to pledge to adhere to a code of conduct that rejects any form of predatory behaviour. However, a few of its members have been very controversial. For example, Dove Medical Press had been admitted as a member, only to be rejected later (Berger and Cirasella, 2015). One of its members, the Swiss publishing company MDPI (Multidisciplinary Digital Publishing Institute) has been the most active opponent to Beall's lists (Beall, 2017). MDPI publishes over two-hundred OA journals. It recently discontinued ten journals like the *Journal of Cybersecurity and Privacy*. Some of its journals remain in the news (Haspelmath, 2013; Rittman, 2015; de Vrieze, 2018).

The Committee on Publication Ethics (COPE) is a trade association to define best practice in the ethics of scholarly publishing. It has a much larger membership of publishers than OASPA. The list of its members is online. While Dove Medical Press is not a member of OASPA, Dove Medical Press is a member of COPE with a 119 OA journals.

8.5.4 White lists of journals

The Directory of Open Access Journals (DOAJ) started out in 2002 at the University of Lund. Since 2012, it is now run by the British charitable company Infrastructure Services for Open Access (IS4OA). In 2018, its database contained some 12,277 journals. After the manuscript sting experiment of Bohannon (2013) that showed that 73 out of 158 (46 %) of DOJA's journals did not have a functional peer review system, DOJA removed over one hundred journals from its database and revamped its criteria for inclusion (Berger and Cirasella, 2015). After 2014, journals subjected to stricter riles gained a green sticker with a tick symbol, journals that adhere to outstanding best practice carry now a Seal in the list. The list is now a mixture of lower criteria, higher criteria with a tick, and a handful of journals of an extra high and clear commitment to OA best practice. DOJA, unfortunately, has still its problems (Clark and Smith, 2015). This became clear when the editor sting experiment revealed that 8 journals out of 120 of DOAJ's whitelist appointed the fake editor, whereas Web of Science listed journals accepted none of Szust's

applications (Sorokowski et al., 2017). If one interpolates 7 % on the total size of DOAJ database, it is a large number of likely predatory journals remaining in the database.

Web of Science and Scopus are expensive databases. The journal lists of Clarivate Analytics (Web of Science) and Scopus can be freely searched online. The list of journals indexed in Web of Science is the basis for the traditional impact factor. Web of Science and Scopus use conservative criteria to select the journals they cover (De Moya-Anegón et al., 2007). However, no system or list is perfect. The journal lists of Web of Science and Scopus do contain some journals that are regarded as predatory (Dadkhah et al., 2017b). Fortunately, that is not the case for forensic journals.

Google Scholar, on the other hand, is completely free. Google Scholar also allows academics to list their publications on their website, to follow people's publications and to be followed. It keeps track of the citations of all the publications in one's list. With these citations, it calculates an h-index and i10 index for each person. The h-index or Hirsch number says that an author has published h number of publications that have been cited at least h-times, for example h = 37 means 37 publications that each have at least attracted 37 citations each. The i10 index gives the number of publications of an author with at least 10 citations. In order to maximise the h- and i10-indices, Google Scholar adds predatory journals and PDF files that seem to be academic documents on the web that carry references to its database (Harzing and Van der Wal, 2008; Delgado et al., 2014; Gutierrez et al., 2015; Beall, 2016; Byard, 2016; Dadkhah et al., 2017b). This means that for forensic work, Google Scholar should be used with extreme caution.

There is another free database of journals. This is MEDLINE, which is maintained by the US National Library of Medicine. It covers biomedical and life sciences journals back to 1946. A National Institutes of Health (NIH)-chartered advisory committee, the Literature Selection Technical Review Committee (LSTRC), reviews and recommends journals for MEDLINE, which currently includes more than 5,200 scholarly journals. MEDLINE and PubMed should not be confused with

each other. PubMed contains all MEDLINE indexed journals but incorporates many more sources from.

Research Square is the parent company of American Journal Experts which provides academic language and manuscript services, among which are recommendations for the most appropriate journal outlet for a given manuscript. For this, it developed JournalGuide, which is a free tool (website) that recommends and also verifies journals based on 40,000 journal profiles, including forensic journals (Mudrak, 2015). The verification is based on being indexed by Scopus as a universal database and specialist databases such as EconLit and MEDLINE.

While almost all predatory journals are new, on rare occasions a publisher has bought up an established journal and turned it predatory (Spears, 2014).

Cabells Scholarly Analytics is a new company erected by management professor David W. E. Cabell to provide black and white lists to the industry and author services by advicing authors in which journals to publish. Cabell's has a total of 11,112 journals for all disciplines in its commercial white lists, for Mathematics, Biological Sciences, Physics, Astronomy, Chemistry, Geology, and Oceanography it lists 4,331 journals.

For forensic journals, there is a specialist database, the Criminal Justice Abstracts, provided by EBSCO Information Services, which indexes more than 600 journals; its journal list can be downloaded.

8.5.5 The third force

Until the recent explosion of number of articles and number of scholarly journals, academic publishing was subjected to two major forces, the reader and the author with the journal editors representing the publisher in the middle of the two. The explosion has made the retrieval of information without databases almost impossible. This has created a third force which acts as an easily overlooked gatekeeper who determines which information or which journals may enter the databases and can be retrieved and which journals are excluded. In part, this gatekeeper role has been exercised by libraries. While a librarian of a university was/is in reach of a scientist and easily identified as a concrete person, journal

selection at a commercial enterprise or at a National library is much more anonymous and far removed from a direct influence of local scientists. The third force now acts directly through the databases themselves and indirectly at trade associations and at professional bodies of the subject field.

8.6 Methods

8.6.1 Journal evaluation

Over a period of four years, a systematic search was undertaking to identify all print and electronic (online) journals covering biological aspects of forensic sciences and legal medicine as their main subject area. For the evaluation of journals, four documents were considered:

- Beall J (2015) Criteria for Determining Predatory Open-Access Publishers. 3rd edition.
- Committee on Publication Ethics (2015) Code of Conduct for Journal Publishers.
- Committee on Publication Ethics (2015) Principles of Transparency and Best Practice in Scholarly Publishing.

and since 2017, in addition,

- Rele S, Kennedy M and Blas N (2017) Journal Evaluation Tool. LMU Librarian Publications & Presentations. Loyola Marymount University, Los Angeles, California, USA

After the individual evaluation of all journals, the journals were assigned to three categories:

- Core Journals
- Specialised, National, and New (SNN) journals: English-language journals
- Specialised, National, and New (SNN) journals: Foreign- and mixed language journals

8.6.2 Peer assessment

The resulting three lists were sent to a panel of forensic scientists for peer assessment. The panel consists of

- Dr. Jens Amendt, Institut für Rechtsmedizin, Forensische Biologie/Entomologie, Goethe University Frankfurt, Frankfurt am Main, Germany
- Dr Henk R Braig, School of Natural Sciences, Bangor University, Bangor, Wales, UK, and supervisor of this thesis
- Assoc. Prof. Jason H. Byrd, William R. Maples Center for Forensic Medicine, Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, Florida, USA
- Prof. M. Lee Goff, Criminal; Justice Hawaii Pacific University, Chaminade University Forensic Sciences Program, Honolulu, and University of Hawaii, Manoa, Hawaii, USA
- Assoc. Prof. M. Alejandra Perotti, School of Biological Sciences, University of Reading, UK
- Prof. Marta I. Saloña Borads, University of the Basque Country, UPV EHU, Bilbao, Spain
- Prof. Jeffery K. Tomberlin, Forensic and Investigative Sciences Program, Department of Entomology, Texas A&M University, College Station, Texas, USA

8.7 Established forensic science journals

The most important part of a journal is the scientific quality of its articles. No one is better suited to judge this quality than the workers, researchers, and practitioners in that field. Based on the quality of the articles and the lack of indicators for predatory behaviour discussed earlier, a white list of current research journals covering biological aspects of forensic sciences and legal medicine forensic journals is proposed in Table 8.1.

List of current research journals covering biological aspects of forensic sciences and legal medicine

Core Journals

Journal	Start	Affiliation	Publisher
American Journal of Forensic Medicine and Pathology	1980	(National Association of Medical Examiners, USA)	Wolters Kluwer
Australian Journal of Forensic Sciences	1968	Australian and New Zealand Forensic Society, Australian Academy of Forensic Sciences, Australia	Taylor & Francis
Forensic Science International (Forensic Science)	1972	–	Elsevier
Forensic Science International: Genetics	2007	International Society for Forensic Genetics	Elsevier
Forensic Science International: Genetics Supplement Series	2008	International Society for Forensic Genetics	Elsevier
Forensic Sciences Research	2016	Academy of Forensic Science, Ministry of Justice, China	Taylor & Francis
Forensic Science, Medicine, and Pathology	2005	–	Springer
Forensic Toxicology (Japanese Journal of Forensic Toxicology)	1990	Japanese Association of Forensic Toxicology, Japan	Springer
International Journal of Legal Medicine (Zeitschrift für Rechtsmedizin, Deutsche Zeitschrift für die gesamte gerichtliche Medizin)	1922	International Academy of Legal Medicine	Springer
Journal of Forensic and Legal Medicine (Journal of Clinical Forensic Medicine)	1994	Société Française de Médecine Légale [French Society of Legal Medicine], France	Elsevier

Journal	Start	Affiliation	Publisher
The Journal of Forensic Practice (The British Journal of Forensic Practice)	1999	–	Emerald
Journal of Forensic Sciences	1956	American Academy of Forensic Sciences, (American Society for Testing and Materials), USA	Wiley
Journal of Law and the Biosciences	2014	Duke University, Harvard U. Law School, and Stanford U.	Oxford UP
Journal of Legal Medicine	1979	American College of Legal Medicine, Georgia State U. College of Law's Center for Law, Health & Society, USA	Taylor & Francis
Legal Medicine	1999	Japanese Society of Legal Medicine, Japan	Elsevier
Medicine, Science, and the Law	1960	British Academy for Forensic Sciences	SAGE
Science & Justice (Journal of the Forensic Science Society)	1961	Forensic Science Society, UK	Elsevier

Specialised, National, and New (SNN) journals

English-language journals

Journal	Start	Affiliation	Publisher
American Journal of Forensic Psychology	1983	American College of Forensic Psychology, USA	same
American Journal of Law and Medicine	1975	American Society of Law, Medicine & Ethics, USA	Boston U
Anil Aggrawal's Internet Journal of Forensic Medicine and	2000	–	Anil Aggrawal, India

Journal	Start	Affiliation	Publisher
Toxicology			
Applied Psychology in Criminal Justice	2005	Criminal Justice Center, University, USA	Sam Houston State U.
Arab Journal of Forensic Sciences and Forensic Medicine	2014	Arab Society for Forensic Sciences and Forensic Medicine, Saudi Arabia	Naif Arab University for Security Sciences
Criminal Behaviour and Mental Health	1996	–	Wiley
Criminal Justice and Behavior	1974	International Association for Correctional and Forensic Psychology (American Association of Correctional Psychologists), USA	SAGE
Egyptian Journal of Forensic Sciences	2011	International Association of Law and Forensic Science, Egypt	Springer
Environmental Forensics	2000	International Society of Environmental Forensics, USA	Taylor & Francis
European Journal of Psychology Applied to Legal Context	2009	Sociedad Española de Psicología Jurídica y Forense [Spanish Society of Legal and Forensic Psychology], Asociación Iberoamericana de Justicia Terapéutica [Ibero-American Association of Therapeutic Jurisprudence], Spain	Colegio Oficial de Psicólogos de Madrid, Spain, (Elsevier)
Forensic Science International Supplement Series	2009		Elsevier
Frontiers in Forensic Psychiatry	2011	–	Frontiers
Identification Canada Journal	1977	Canada Identification Society, Canada	same
Indian Internet Journal of Forensic Medicine and Toxicology	2003	Indian Congress of Forensic Medicine & Toxicology, India	same
Indian Journal of Forensic Medicine and Toxicology	2015		Institute of Medico-

Journal	Start	Affiliation	Publisher
(Journal of Forensic Medicine and Toxicology)			Legal Publications, India
Interdisciplinary Toxicology	2008	Institute of Experimental Pharmacology of Slovak Academy of Sciences, Slovakia	De Gruyter
International Journal of Forensic Mental Health	2002	International Association of Forensic Mental Health Services	Taylor & Francis
International Journal of Law and Psychiatry	1978	International Academy of Law and Mental Health	Elsevier
International Journal of Medical Toxicology and Forensic Medicine	2011	Department of Forensic Medicine and Toxicology, Shahid Beheshti U of Medical Sciences, Iran	same
Japanese Journal of Forensic Science and Technology	1996	Japanese Association of Forensic Science and Technology, Japan	J-Stage
Journal of Analytical Toxicology	1977	Society of Forensic Toxicologists, USA	Oxford UP
Journal of Forensic Dental Sciences	2009	Indian Association of Forensic Odontology, Japan	Wolters Kluwer
Journal of Forensic Identification	1969	International Association for Identification, USA	same
Journal of Forensic Nursing	2005	International Association of Forensic Nurses, USA	Wolters Kluwer
Journal of Forensic Odonto-Stomatology (International Journal of Forensic Dentistry)	1983	International Organization for Forensic Odonto-Stomatology, Belgium	EBSCO
The Journal of Forensic Psychiatry and Psychology (The Journal of Forensic Psychiatry)	1990	–	Taylor & Francis
Journal of Forensic Psychology Research and Practice	1900	–	Taylor & Francis

Journal	Start	Affiliation	Publisher
(Journal of Forensic Psychology Practice)			
Journal of Forensic Radiology and Imaging	2013	International Society of Forensic Radiology and Imaging, International Association of Forensic Radiographers	Elsevier
Journal of Forensic Science and Medicine	2015	China University of Political Science and Law, China	same
Journal of Indian Academy of Forensic Medicine	1978	Indian Academy of Forensic Medicine	same
Journal of Law, Medicine and Ethics (Law, Medicine & Health Care, Medicolegal News, Nursing Law & Ethics)	1973	American Society of Law, Medicine & Ethics, USA	Wiley
Journal of Punjab Academy of Forensic Medicine and Toxicology	2001	Punjab Academy of Forensic Medicine and Toxicology	same
Journal of South India Medicolegal Association	2009	South India Medicolegal Association	same
The Journal of the American Academy of Psychiatry and the Law (Bulletin of the American Academy of Psychiatry and the Law)	1973	American Academy of Psychiatry and the Law, USA	same
Legal and Criminological Psychology	1996	British Psychological Society	Wiley
Malaysian Journal of Forensic Sciences	2010	Forensic Science Society of Malaysia	same
Mansoura Journal of Forensic Medicine and Clinical Toxicology	1993	Dept. of Forensic Medicine and Toxicology, Mansoura Faculty of Medicine, Egypt	same
Medical Law Review	1997	–	Oxford UP
Medicolegal and Bioethics	2011	–	Dove
Medico-Legal Journal of Ireland	1995	University College Dublin, Division of	Thomson Round Hall

Journal	Start	Affiliation	Publisher
		Legal Medicine, Ireland	
Medico-Legal Update	1996	–	Institute of Medico-Legal Publications, India
Problems in Forensic Sciences (Z Zagadnień Nauk Sądowych, Z zagadnień kryminalistyki)	1960	Instytut Ekspertyz Sądowych [Institute of Forensic Research in Kraków], Poland	same
Psychology, Crime and Law	1994	European Association of Psychology and Law	Taylor & Francis
Psychiatry, Psychology, and Law	1994	Australian and New Zealand Association of Psychiatry, Psychology and Law	Taylor & Francis
Regulatory Toxicology and Pharmacology	1981	International Society for Regulatory Toxicology and Pharmacology	Elsevier
THEMIS – Research Journal of Justice Studies and Forensic Science	2013	San Jose State University, USA	SJSU ScholarWorks
Romanian Journal of Legal Medicine	1993	Romanian Society of Legal Medicine	same
Scandinavian Journal of Forensic Science	2004	Dansk Selskab for Retsmedicin [Danish Society of Forensic Medicine], Norsk Rettsmedisinsk Forening [Norwegian Society of Forensic Medicine], Svensk Rättsmedicinsk Förening [Swedish Society of Forensic Medicine], Denmark, Norway, Sweden	de Gruyter
Sri Lanka Journal of Forensic Medicine, Science and Law	2010	Department of Forensic Medicine, Faculty of Medicine, University of Peradeniya, Sri Lanka	Sri Lanka Journals online

Foreign- and mixed language journals

Journal	Start	Affiliation	Publisher
Anuario de Psicología Jurídica [Annual Review of Legal Psychology]	1991	Colegio Oficial de Psicólogos de Madrid, Spain	same, (Elsevier)
Archives of Forensic Medicine and Criminology	1951	Polish Society of Forensic Medicine and Criminology, Poland	Termedia
Archivos de Criminología, Criminalística y Seguridad Privada [Archives of Criminology, Criminalistics and Private Security]	2008	Sociedad Mexicana de Criminología capítulo Nuevo León, Mexico	same
Brazilian Journal of Forensic Sciences, Medical Law and Bioethics	2011	Instituto Paulista de Estudos Bioéticos e Jurídicos [São Paulo Institute of Bioethical Studies and Legal], Brazil	same
Canadian Society of Forensic Science Journal	1968	Canadian Society of Forensic Science, Canada	Taylor & Francis
Chinese Journal of Forensic Medicine	1986	Chinese Forensic Medicine Association, China	Zhong guo fa yi xue hui
Ciencia Forense. Revista Aragonesa de Medicina Legal [Forensic Science. Aragonese Journal of Legal Medicine]	1999	Institución Fernando el Católico, Spain	same
Cuadernos de Medicina Forense [Journal of Forensic Medicine]	1995	Asociación de Médicos Forenses de Andalucía [Coroners' Association of Andalusia], Spain	ESMON
Forensische Psychiatrie, Psychologie, Kriminologie [Forensic Psychiatry, Psychology, Criminology]	2007	Deutsche Gesellschaft für Psychiatrie, Psychotherapie und Nervenheilkunde [German Society for Psychiatry,	Springer

Journal	Start	Affiliation	Publisher
		Psychotherapy and Neurology], Sektion Rechtspsychologie im Berufsverband Deutscher Psychologinnen und Psychologen [Section Forensic Psychology of the Professional Association of German Psychologists], Fachgruppe Rechtspsychologie in der Deutschen Gesellschaft für Psychologie [Section Forensic Psychology in the German Psychological Society], Kriminologische Gesellschaft [Criminological Society], Germany	
Journal de Médecine Légale, Droit Médical, Victimologie, Dommage Corporel [Journal of Legal Medicine, Medical Law, Victimology, Personal Injury]	1981	Société de Médecine légale et de Criminologie de France [French Society for Legal Medicine and Criminology], Association Lyonnaise de Médecine Légale [Association of Legal Medecine of Lyon], France	ESKA, Lacassagne
Medicina Legal de Costa Rica [Legal Medicine in Costa Rica]	1984	Asociación Costarricense de Medicina Forense [Association for Legal Medicine], Costa Rica	SciELO
Rechtsmedizin [Legal Medicine]	1997	Deutsche Gesellschaft für Rechtsmedizin [German Society of Legal Medicine]	Springer
Psicologia & Giustizia [Psychology & Justice]	2000	Fondazione Guglielmo Gulotta di Psicologia Interpersonale Investigativa	same

Journal	Start	Affiliation	Publisher
		Criminale e Forense [Guglielmo Gulotta Foundation for Interpersonal Psychology in Criminal and Forensic Investigations], Italy	
Psihologiâ i Pravo [Psychology and Law]	2011	Moscow State University of Psychology and Education, Russia	same
Revista Española de Medicina Legal [Spanish Journal of Legal Medicine]	1974	Asociación Nacional de Médicos Forenses [National Association of Forensic Physicians], Spain	Elsevier
La Revue de Médecine Légale [The Journal of Legal Medicine]	2010	Société Française de Médecine Légale [French society of Legal Medicine], France	Elsevier
Rivista di Psicopatologia Forense, Medicina Legale, Criminologia [Journal of Forensic Psychopathology, Legal Medicine, Criminology]	1960	–	PAGEPress, Italy
Scientific Journal of Forensic Medicine	2000	Organization of Legal Medicine of the Islamic Republic of Iran, Iran	same
Soudní lékařství [Forensic Pathology]	1956	Československá Lékařská Spolecnost J. Ev. Purkyně, Sekce Soudního Lékařství [Czechoslovak Medical Association J. Ev. Purkyně, Section of Forensic Medicine], Česká Společnost Soudního Lékařství a Soudní Toxikologie [Czech Society of Forensic Medicine and Toxicology],	Státní zdravotnické nakladatelství

Journal	Start	Affiliation	Publisher
		Společnost Soudního Lékařství [Society of Forensic Medicine], Czech Republic	
Sudebno-Meditsinskaya Ekspertisa [Forensic Medical Examination]	1958	–	Izdatel'stvo Meditsina, Russia
Zhongguo Fa Yi Xue Za Zhi [Chinese Journal of Forensic Medicine]	1986	–	Si fa bu, China

Table 8.1 – Proposal of a white list for research journals covering biological aspects of forensic sciences and legal medicine.
Journal names and affiliations in parentheses are former names and affiliations. Translations are in brackets.
The list focuses on journals with an emphasis on forensic biology and legal medicine. Journals that have a much wider remit have not been included, such as Aggression and Violent Behavior, Bulletin on Narcotics, Child Abuse & Neglect, Child Maltreatment, International Journal of Biological Markers, Forensic Science: Policy and Management, Journal of Child Sexual Abuse, Journal of Interpersonal Violence, Journal of Tissue Viability, Violence and Victims, and all journals of general pathology or medical entomology and medical microbiology.
Magazines, professional news journals, annual reports, pure review journals and related journals such as CAC News, Crime Lab Minute, Crime Lab Report, Evidence Technology Magazine, FBI Laboratory Annual Report, Forensic Drug Abuse Advisor, Forensic Magazine, Forensic Science Review, Global Forensic Science Today, Kriminalistik, Legal Medicine Annual, National Forensic Journal, Police Chief, Royal Canadian Mounted Police Gazette, Scientific Testimony, and so on have not been included.
Journals covering mainly criminological aspects or have a focus on policing or ethics such as Agora International Journal of Juridical Sciences, Australian and New Zealand Journal of Criminology, British Journal of Criminology, Crime and Delinquency, Crime, Crime Science, Criminology, Criminology & Social Integration Journal, Duke Journal of Gender Law and Policy, Droit, Déontologie et Soin [Law, Deontology and Care], FBI Law Enforcement Bulletin, Homicide Studies, International Criminal Justice Review, International Journal of Offender Therapy and Comparative Criminology, Internet Journal of Law, Healthcare and Ethics, Journal of Contemporary Criminal Justice, Journal of Criminal Investigation and Criminology, Journal of Criminal Law and Criminology, Journal of Criminal Justice, Journal of Criminology, Journal of Quantitative Criminology, Journal of Research in Crime and Delinquency, Justice Quarterly, Law and Social Change, Pittsburgh Journal of Environmental and Public Health Law, Policing, Revista CENIPEC, Revista Española de Sanidad Penitenciaria [Spanish Journal of Penitentiary Health], Theoretical Criminology, and so on have not been included. In German, the similar looking terms and journals Kriminalistik (English: mainly forensics) and Kriminologie (English: criminology) have different meanings and coverage. At the edges where these fields overlap, a personal choice decided upon the inclusion in the list.

8.8 Challenges ahead

Encourage members of professional societies to refrain from publishing in predatory journals. This might be accomplished through information on all levels. Professional societies might help members to quit or fight editorial memberships.

Stop citing papers in predatory journals is a much greater challenge that currently lacks any technical underpinning.

8.9 References

Beall J (2012) Predatory publishers are corrupting open access. *Nature* 489: 179.

Beall J (2013) Medical publishing triage—Chronicling predatory open access publishers. *Annals of Medicine and Surgery* 2: 47–49.

Beall J (2016) Predatory publishers threaten medical research. *Journal of Korean Medical Science* 31: 1511-1513.

Beall J (2017) What I learned from predatory publishers. *Biochemia Medica* 27: 273–279.

Berger M and Cirasella J (2015) Beyond Beall's list: Better understanding predatory publishers. *College & Research Libraries News* 76: 132-135.

Bohannon J (2013) Who's afraid of peer review? *Science* 342: 60-65.

Bohannon J (2015a) How to hijack a journal. *Science* 350: 903–905.

Bohannon J (2015b) Hoax-detecting software spots fake papers. *Science* 348: 18-19.

Butler D (2013a) Sham journals scam authors. *Nature* 495: 421-422.

Butler D (2013b) Investigating journals: The dark side of publishing. *Nature* 495: 433-435.

Byard RW (2016) The forensic implications of predatory publishing. *Forensic Science, Medicine, and Pathology* 12: 391-393.

Clark J and Smith R (2015) Firm action needed on predatory journals. *British Medical Journal* 350: h210.

Clemons M, de Costa E, Silva M, Joy AA, Cobey KD, Mazzarello S, Stober C and Hutton B (2017) Predatory invitations from journals: More than just a nuisance? *Oncologist* 22: 236–240.

Dadkhah M, Maliszewski T and Teixeira da Silva JA (2016) Hijacked journals, hijacked web-sites, journal phishing, misleading metrics, and predatory publishing: Actual and potential threats to academic integrity and publishing ethics. *Forensic Science, Medicine, and Pathology* 12: 353–362.

Dadkhah M, Borchardt G and Maliszewski T (2017a) Fraud in academic publishing: Researchers under cyber-attacks. *American Journal of Medicine* 130: 27-30.

Dadkhah M, Lagzian M and Borchardt G (2017b) Questionable papers in citation databases as an issue for literature review. *Journal of Cell Communication and Signaling* 11: 181-185.

Daubert and Merrell Dow Pharmaceuticals (1993) *Daubert v. Merrell Dow Pharmaceuticals, Inc., 509 U.S. 579 (1993)*.

De Moya-Anegón F, Chinchilla-Rodríguez Z, Vargas-Quesada B, Corera-Álvarez E, Muñoz-Fernández F, González-Molina A and Herrero-Solana V (2007) Coverage analysis of Scopus: A journal metric approach. *Scientometrics* 73: 53–78.

de Vrieze J (2018) Open-access journal editors resign after alleged pressure to publish mediocre papers. *Science*: 10.1126/science.aav3129.

Delgado LE, Robinson-Garcia N and Torres-Salinas D (2014) The Google Scholar experiment: How to index false papers and manipulate bibliometric indicators. *Journal of the Association for Information Science and Technology* 65: 446–454.

Dewan P and Shah D (2016) A writer's dilemma: Where to publish and where not to? *Indian Pediatrics* 53: 141–145.

Else H (2018) Radical open-access plan could spell end to journal subscriptions. *Nature* 561: 17-18.

Gasparyan AY, Yessirkepov M, Diyanova SN and Kitas GD (2015) Publishing ethics and predatory practices: A dilemma for all stakeholders of science communication. *Journal of Korean Medical Science* 30: 1010–1016.

Gasparyan AY, Nurmashev B, Voronov AA, Gerasimov AN, Koroleva AM and Kitas GD (2016) The pressure to publish more and thescope of predatory publishing activities. *Journal of Korean Medical Science* 31: 1874–1878.

Gutierrez FR, Beall J and Forero DA (2015) Spurious alternative impact factors: the scale of the problem from an academic perspective. *Bioessays* 37: 474-476.

Hansoti B, Langdorf MI and Murphy LS (2016) Discriminating between legitimate and predatory open access journals: Report from the International Federation for Emergency Medicine Research Committee. *Western Journal of Emergency Medicine* 17: 497–507.

Harzing AWK and Van der Wal R (2008) Google scholar as a new source for citation analysis. *Ethics in Science and Environmental Politics* 8: 61–73.

Haspelmath M (2013) Why open-access publication should be nonprofit—A view from the field of theoretical language science. *Frontiers in Behavioral Neuroscience* 7: 57.

Jalalian M and Dadkhah M (2015) The full story of 90 hijacked journals from August 2011 to June 2015. *Geographica Pannonica* 19: 73–87.

Johal J, Ward R, Gielecki J, Walocha J, Natsis K, Tubbs RS and Loukas M (2017) Beware of the predatory science journal: A potential threat to the integrity of medical research. *Clinical Anatomy* 30: 767-773.

Kannan M (2015) Beware of predatory journals. Know thy publisher. *Lab Times* 2015-3: 18-21.

Kearney MH and The INANE Predatory Publishing Practices Collaborative (2015) Predatory publishing: what authors need to know. *Research in Nursing and Health* 38: 1-3.

Kebede M, Schmaus-Klughammer AE and Tekle BT (2017) Manuscript submission invitations from 'predatory journals': What should authors do? *Journal of Korean Medical Science* 32: 709–712.

Knoll JL (2014) Open Access journals and forensic publishing. *Journal of the American Academy of Psychiatry and the Law* 42: 315-321.

Labbe C and Labbe D (2013) Duplicate and fake publications in the scientific literature: How many SCIgen papers in computer science? *Scientometrics* 94: 379-396.

Maddy AJ and Tosti A (2017) Predatory journals in dermatology. *British Journal of Dermatology* 177: 307-309.

Marcus A and Oransky I (2016) Why fake data when you can fake a scientist? Making up names and CVs is one of the latest tricks to game scientific metrics. *Nautilus* 2016: November 24.

Masic I (2017) Predatory publishing - Experience with OMICS International. *Medical Archives* 71: 304-307.

Moher D and Srivastava A (2015) You are invited to submit …. *BMC Medicine* 13: 180.

Mudrak B (2015) JournalGuide: Bringing authors and journals together. *Learning & Behavior* 28: 147-149.

New J (2013) Publisher threatens to sue blogger for $1 billion. *The Chronicle of Higher Education* May 15.

Nicoll LH and Chinn PL (2015) Caught in the trap: The allure of deceptive publishers. *Nurse Author and Editor* 25: e4.

Noga-Styron KE, Olivero JM and Britto S (2017) Predatory journals in the criminal justices sciences: Getting our cite on the target. *Journal of Criminal Justice Education* 28: 174-191.

Norko MA (2014) Introduction to the special section on forensic publishing: An examination. *Journal of the American Academy of Psychiatry and the Law* 42: 278-281.

Oury C (2017) Assessing the ISSN register: Defining, evaluating, and improving the quality of a shared international bibliographic database. *Cataloging & Classification Quarterly* 55: 588-605.

Peternelj-Taylor C (2015) What authors need to know about predatory publishing. *Journal of Forensic Nursing* 11: 1–3.

Rittman M (2015) Commentary: "Why open-access publication should be nonprofit-a view from the field of theoretical language science". *Frontiers in Behavioral Neuroscience* 9: 201.

Shamseer L, Moher D, Maduekwe O, Turner L, Barbour V, Burch R, Clark J, Galipeau J, Roberts J and Shea BJ (2017) Potential predatory and legitimate biomedical journals: can you tell the difference? A cross-sectional comparison. *BMC Medicine* 15: 28.

Shen C and Björk B-C (2015) 'Predatory' open access: a longitudinal study of article volumes and market characteristics. *BMC Medicine* 13: 230.

Shoucair EFE (2018) Cabining judicial discretion over forensic evidence with a new special relevance rule. *Michigan Law Review* 117: 173-196.

Sorokowski P, Kulczycki E, Sorokowska A and Pisanski K (2017) Predatory journals recruit fake editor. *Nature* 543: 481–483.

Spears T (2014) Canadian cardiac journal turns "Predatory". *Canadian Medical Association Journal* 188: e525.

Stojanovski J and Marušić A (2017) Does small equal predatory? Analysis of publication charges and transparency of editorial policies in Croatian open access journals. *Biochemia Medica* 27: 292–299.

Tosti A and Maddy AJ (2017) Ranking predatory journals in dermatology: Distinguishing the bad from the ugly. *International Journal of Dermatology* 56: 718-720.

Xia J (2015) Predatory journals and their article publishing charges. *Learned Publishing* 28: 69-74.

Xia J and Smith MP (2018) Alternative journal impact factors in open access publishing. *Learned Publishing* 31: 403-411.

9 Conclusions and outlook

9.1 Forensic biology in Arabian countries

The literature on forensic biology from Arabian countries shows impressive body of research and data on forensic entomology, increasingly using insects estimating for estimation of post-mortem intervals of human corpses. Insects involved in human and animal decomposition are more and more identified by their molecular barcode.

However, forensic biology has so far not been used in a single case as trace evidence. While mites have been identified in a few forensic studies on the species succession during decomposition of modal animal carcasses, the full potential of forensic acarology still awaits its full development.

9.2 Dust as forensic trace evidence

Microsatellites are one of the most informative and versatile DNA-based markers that have been widely and successfully used in population and conservation genetic studies. The same holds true for the application of microsatellites in forensics. The biggest limitation to the use of microsatellites has been its development for any species being laborious, time-consuming, and expensive. Here, whole genome analysis of mites has been used only for the second time to develop unbiased microsatellites from scratch.

At the heart of most forensic work lies the establishment of identity through linking characteristics or characters of any sort with a person, organism or object. The physical, mineral, and chemical characters of dust are fully explored in forensic work. The biological characters, especially in the form of dust mites, have so far been overlooked. The identity of dust mites can be most easily obtained with the help of microsatellites, despite the fact that the application of microsatellites to the analysis and characterisation of the ever-increasing diversity of non-human biological trace samples is still in its infancy. As far as humidity allows, dust mites have great value and potential as biological race evidence in forensics.

9.3 Microsatellites in forensics

For the first time, the full, unbiased diversity of microsatellites has been investigated in an Acari genome. While a previous study started investigating perfect

microsatellites of eleven and more repeats of di-, tri-, quatro- and penta nucleotides, this study included all di- to decanucleotides of any repeat length and including imperfect microsatellites as well.

What is now needed is either a small army of master students or a PhD student to collect dust/dust mites and apply these microsatellites to as many different 'populations of dust' as possible. The two most common dust mites worldwide are *D. farinae* and *D. pteronyssinus* because of their close association with humans (Klimov et al., 2019). It will be very interesting to estimate the population size of both species with the now available microsatellites. It might be possible that *D. farinae* is one of the animal species with largest population size known.

The future will certainly involve developing microsatellites directly from whole genomes, as has been done here (Mokhtar and Atia, 2019). Most genomic sequencing is not whole genome sequencing but transcriptome or RNA sequencing and therefore limits the discovery of microsatellites to genic microsatellites.

For the most time it has been assumed that non-genic microsatellites are less restricted, but recent research has shown an increasing number of non-genic microsatellites actually do have function in the regulation of the genome (Bagshaw, 2017). This also means that non-genic microsatellites might be more restricted than assumed so far. An early study on genic and non-genic microsatellites recommends to combine both for optimal resolution (DeFaveri et al., 2013).

Currently microsatellites are the preferred molecular marker. This will change as soon as the sensitivity of other methods can be increase, for example, with the routine application of whole genome amplification to each forensic trace sample. This would see the replacement of microsatellites with newer methods (Hodel et al., 2016) that do no longer require development for individual species. It is highly likely that sooner or later one of the many reduced-representation sequencing methods like restriction site associated DNA sequencing (RADseq) will replace microsatellites in biological non-human forensic trace evidence, including mites (Xue et al., 2017).

The current work has exposed the dangers of using microsatellites in metagenomic studies involving closely related species, especially in a forensic context.

9.4 Integrity of forensic data

The outlook in the fight against false data and predatory journals is bleak at least. Only a very few University libraries like the one of Yale University pay attention to the problem. There is no evidence that the libraries of the Universities in the UK are even aware of the problem. Many University librarians recommend students to use Google Scholar which includes man predatory journals. Despite the huge amount of money that goes into predatory publishing, the non-predatory publishing industry does not see a way to take advantage of it, which means it has little incentive to help the fight against predatory journals. The citation of predatory articles will sneak into every aspect of science unnoticed. At the same time, the predatory publishers will gain ever more economic power. Anyone trying to oppose, will face complains and threats to the University with the danger of losing one's job. Working on something that points out mistakes in others is everything but personally rewarding or satisfactory. For forensics, the problem will be exacerbated in countries that do not have controversial legal system. Predatory journals might be at the heart of miscarriages of justice.

9.5 References

Bagshaw ATM (2017) Functional mechanisms of microsatellite DNA in eukaryotic genomes. *Genome Biology and Evolution* 9: 2428-2443.

DeFaveri J, Viitaniemi H, Leder E and Merila J (2013) Characterizing genic and nongenic molecular markers: comparison of microsatellites and SNPs. *Molecular Ecology Resources* 13: 377-392.

Hodel RDGJ, Segovia-Salcedo MC, Landis JB, Crowl AA, Sun M, Liu X, Gitzendanner MA, Douglas NNA, Germain-Aubrey CC, Chen S, Soltis DE and Soltis PS (2016) The report of my death was an exaggeration: A review for researchers using microsatellites in the 21st century. *Applications in Plant Sciences* 4: e1600025.

Klimov PB, Skoracki M and Bochkov AV (2019) Cox1 barcoding versus multilocus species delimitation: Validation of two mite species with contrasting effective population sizes. *Parasites and Vectors* 12: e8.

Mokhtar MM and Atia MAM (2019) SSRome: An integrated database and pipelines for exploring microsatellites in all organisms. *Nucleic Acids Research* 47: D244-D252.

Xue D-X, Li Y-L and Liu J-X (2017) A rapid and cost-effective approach for the development of polymorphic microsatellites in non-model species using paired-end RAD sequencing. *Molecular Genetics and Genomics* 292: 1165-1174.