House dust mites and their genetic systems

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Declaration

Declaration:

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged

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Abstract

Despite their medical implications and widespread distribution, there is limited knowledge of house dust mites. I assessed their biogeographical distribution, examined the genetic system of one of the most common species of house dust mite, *Dermatophagoides farinae*, and modelled different mating systems of mites, with the aim of improving understanding. This included assembling a database of house dust mite diversity, and identifying the most successful protocols for molecular work, which will save researchers time and provide a solid base for future studies. The creation of a house dust mite fauna database through published resources provided a worldwide distribution of all mites collected from homes. This may prove useful in future, particularly by allergists who are focusing on eradicating these mites for the benefit of human health. In addition, this identified the possibility of a latitudinal diversity gradient in house dust mites. Assessing *D. farinae* mitochondrial and nuclear genome regions through PCR and sequencing has illustrated dissimilarity between populations. This suggested that *D. farinae* may have been previously misidentified and the examined populations actually represent more than one species. This gives a basis for further analysis with an increased number of populations from a variety of locations. Finally, modelling and comparing different mating systems which may be found in mites, illustrate that despite the benefits of being haplodiploid, it is difficult to transition to this system from diploidy. This indicates that cytoplasmically inherited maternally-transmitted haplodiploidy is not favoured in diploid populations, forming part of the reason why many species are still in the ancestral diploid state.

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Chapter 1: Knowledge and Applications of House Dust Mites

The Acari are an underrepresented taxonomic group with a limited amount of knowledge regarding taxonomy, sequence data, and genetic information (Weeks et al., 2000). Mites are very small, there is incomplete knowledge regarding their distribution, and similar species are often confused and misidentified (O'Connor, 2009, Braig & Perotti, 2009, Perotti et al., 2009). This dissertation will begin by broadly discussing some of the more widely-studied mites, as well as some of their known applications and implications to humans (Chapter 1). Subsequently, focus will be narrowed specifically to the house dust mites (HDMs) and their presence worldwide in the published literature (Chapters 2 and 3), with a particular focus on the American house dust mite, *Dermatophagoides farinae* and molecular techniques (Chapters 4 and 5). Finally, computer simulations of some of the different genetic systems which are present in mites will be examined (Chapters 6 and 7), before future directions for this research are outlined (Chapter 8).

Chapter 1 will firstly provide a basic introduction regarding house dust mites, before outlining the taxonomy of mites, including a description of species which have been sequenced thus far. The importance of mites for economic, medical, and forensic purposes will be described, as well as discussing a variety of mating systems which have been identified in different species of mites.

1.1 Ecology of House Dust Mites

1.1.1 Mites in the home:

House dust mites are micro-arthropods, which feed on shed skin scales and other organic debris collected from house dust (Arlian & Platts-Mills, 2001). The small size of mites enables them to exist in many microhabitats, therefore species and number of mites can vary between different sampled locations within a home (Frost et al., 2010). The composition of mite fauna in any given location is influenced by many factors including temperature, altitude, light, and relative air humidity (van Bronswijk, 1981). Temperate climates are more likely to have one dominant species collected from a single dust sample, whereas tropical climates have a minimum of three predominant species (Colloff, 2009). *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Euroglyphus maynei* comprise 90% of mite fauna in a home (Blythe et al., 1974), and their ability to survive in different climates and microenvironments mean they may not be collected from the same location within the home (Crowther et al., 2000).

1.1.2 Life cycle:

There are five distinct life stages of pyroglyphid mites: egg, larva, protonymph, tritonymph, and adult (van Bronswijk & Sinha, 1971). *D. farinae* mites prefer a higher temperature than some other species of HDMs (van Bronswijk & Sinha, 1971). This species dehydrates after death and can comprise up to 80% of the mite population in household mattresses (Crowther et al., 2000). Due to an extended post egg period, *D. farinae* females are able to live longer than *D. pteronyssinus* (100 v 31 days) (Crowther et al., 2000, Arlian & Dippold, 1996). Laboratory experiments have demonstrated that these mites are capable of multiple matings; although the second batch of fertilized eggs are smaller and have a shorter reproductive period

(18 days v. 34 days) (Alexander et al., 2002). *D. farinae* mites have high survival when reared at continuous 75% RH for ten weeks (Arlian et al., 1999).

1.1.3 Humans in the home:

Humans have the largest effect on mite fauna, as they are responsible for controlling different factors in the home (ventilation, temperature, humidity, and furnishings) (van Bronswijk, 1981). General household activities by the inhabitants of a house (ie cleaning or making the bed), has an impact on the dust structure and competition between mite species (Cunnington & Gregory, 1968, Takaoka et al., 1977a). In addition, mites are transferred between locations in the home with the assistance of the inhabitants (Colloff, 2009, Perotti & Braig, 2009), and the dead skin cells shed from humans act as a food source for HDMs (van Bronswijk, 1981). Although space and predation is often a limiting factor with population size, it is most likely that the food supply of dead skin shed by a person per day contributes most to the size of HDM populations within their natural environment (Crowther et al., 2000).

1.1.4 Pets in the home:

The domestication of animals also had an impact on mite fauna, with *Cheyletiella* a common parasitic presence in cats, dogs and rabbits (Dobrosavljevic et al., 2007). Feather mites may be brought in by birds, and lizards and snakes are commonly associated with mites from the families Pterygosomatidae and Omentolaelapidae (Frost et al., 2010). Demodicidae in the hair follicle of humans, as well as dogs, cats, gerbils, and hamsters illustrate harmonious coexisting between species (Frost et al., 2010). Homes with a dog were found to have a higher incidence rate of *Blomia tropicalis*, *Cheyletus* sp., and *Gohieria fusca* (Baqueiro et al., 2006). Finally, homes with predominately indoors pets are correlated with an increased quantity of animal dander, which leads to higher mite density due to increased food sources (Binotti et al., 2005).

1.1.5 Colonisation of mites:

HDMs are collected from a variety of microenvironments within the home, often being transferred indoors on pets, humans, furniture, stored food products and commercial pet foods. Mites continue to disperse around the home, most frequently by passive transport on human clothing (Tovey et al., 1995). Tovey *et al*. (1995) examined living mites and mite allergen levels from individuals in Sydney, Australia with high quantities of mite allergens identified on all items of clothing. Mites were isolated from a sub-sampling of 8 of 15 items of clothing, with woollen clothing harbouring the most mites and allergen levels due to infrequent washing (Tovey et al., 1995). Another study used marked *D. farinae* mites stained with Sudan Red 7B dye and acetone to examine dispersal within a two-story house (Mollet & Robinson, 1996). Approximately 1850 marked mites were released on a downstairs sofa and left overnight. The following day two young children sat on the sofa for three hours, and then their clothes were removed and 51 live marked mites were recovered, as well as unmarked mites. Upholstered surfaces in the house were sampled 10 days later with both marked and unmarked mites collected in small numbers, illustrating their active dispersal around the house (Mollet & Robinson, 1996).

As mites are transferred between locations on individuals' clothing, there is the potential for mites which normally reside in barns or in farm storage locations to be brought into farmhouses. For example, mites were collected from 86.4% of 121 farms across five regions in Germany, with a total of 49 different species identified (Franz et al., 1997). Furthermore, 12 species of mites were identified from stored hay on barn floors of 30 farms in the Swedish island of Gotland (Böstrom et al., 1997), and 70 species of mites were collected from grain storages, handling areas, and stock rearing facilities from 31 Scottish farms (Jeffrey, 1976).

Mites may also be dispersed within the home via stored food products, illustrated by an investigation into the levels of mite-contaminated food imported through southern California ports (Olsen, 1983). This included 35 types of food from 12 different countries, with 212 positive samples contaminated by 22 mite species. Living mites or surviving eggs which hatch after the product was sold and taken into the home, would then be able to disperse (Olsen, 1983). Under laboratory settings, *D. farinae* and *Glycyphagus domesticus* can survive at varying levels when 41 different types of common cereals, cereal product + human food, and animal food were provided as a food source (Sinha & Paul, 1972). Further research illustrated that storage mites easily contaminate unopened commercial dry dog food from manufacturing factories, representing an additional manner mites contaminate the home (Brazis et al., 2008).

Human habitation is often necessary for the presence of HDMs, exemplified by the lack of mites collected from newly built, uninhabited homes in Europe (van Bronswijk, 1981). Only one of 26 sampled houses in Ohio, USA did not contain HDMs, as it had been constructed less than two months prior and had all new furniture and carpeting (Arlian et al., 1982). Additional studies in The Netherlands identified mites on the floors of homes almost immediately after humans occupy a house (van Bronswijk, 1974). However almost all mites were dead and collected in small numbers, indicating they were transferred into the new home on clothing and furniture from their previous location (van Bronswijk, 1974). Living mites were only collected from floors of homes in The Netherlands and Japan after humans had occupied the beds and furniture for at least one week (van Bronswijk, 1974, Miyamoto & Ouchi, 1976). Very few mites were found in homes in Colorado, USA; however, houses containing furniture imported from California, Texas, Tennessee and Germany had a higher mite population due to more humid climates (Moyer et al., 1985). Mite population densities then decreased over the next two years until the normal mite density of Colorado homes was reached (Moyer et al., 1985).

1.2 Taxonomy of Mites

1.2.1 Accuracy:

Many difficulties arise with accurate taxonomic classification of mites, due to outdated and differing nomenclature. Although certain aspects of taxonomy appears to be consistent among acarologists (Kingdom: Animalia, Phylum: Arthropoda, Class: Arachnida, and Subclass: Acari (or Acarina)), ordinal / subordinal classification is not consistent between mite species (Colloff, 1998). There is limited fossil evidence of terrestrial Arthropoda due to their small size and lack of a large exoskeleton, however there are some estimations of species divergence (Krantz & Walter, 2009). Despite these discrepancies, there are two distinct taxa within the Acari: Acariformes and Parasitiformes (Krantz & Walter, 2009).

1.2.2 The Acariformes:

Modern-day descendants of the Acariformes feed on fungi, algae, and organic soil debris, and likely invaded land through the soil pores of the littoral zone (Krantz & Walter, 2009). The Acariformes are subcategorised into the Trombidiformes and Sarcoptiformes, however inconsistent taxonomy results in them being listed as either an order or suborder (Colloff, 1998, Krantz & Walter, 2009). Furthermore, four main groups have been identified within this order: Astigmata, Endeostigmata, Oribatida (Cryptostigmata), and Trombidiformes (Prostigmata) (Krantz & Walter, 2009). The limited fossilisation record has made accurate paleontological dating difficult, however it is likely that the Trombidiformes, Endeostigmata, and Oribatida originated much earlier (410-415 MYA) than the Astigmata (370 MYA) (Dabert et al., 2010). No further details are given to the divergence of these suborders on TimeTree, besides an estimated time of 494 MYA (Kumar et al., 2017). Figure 1.1 illustrates the taxonomic tree of members of the Acariformes with published genomes on NCBI GenBank (2017).

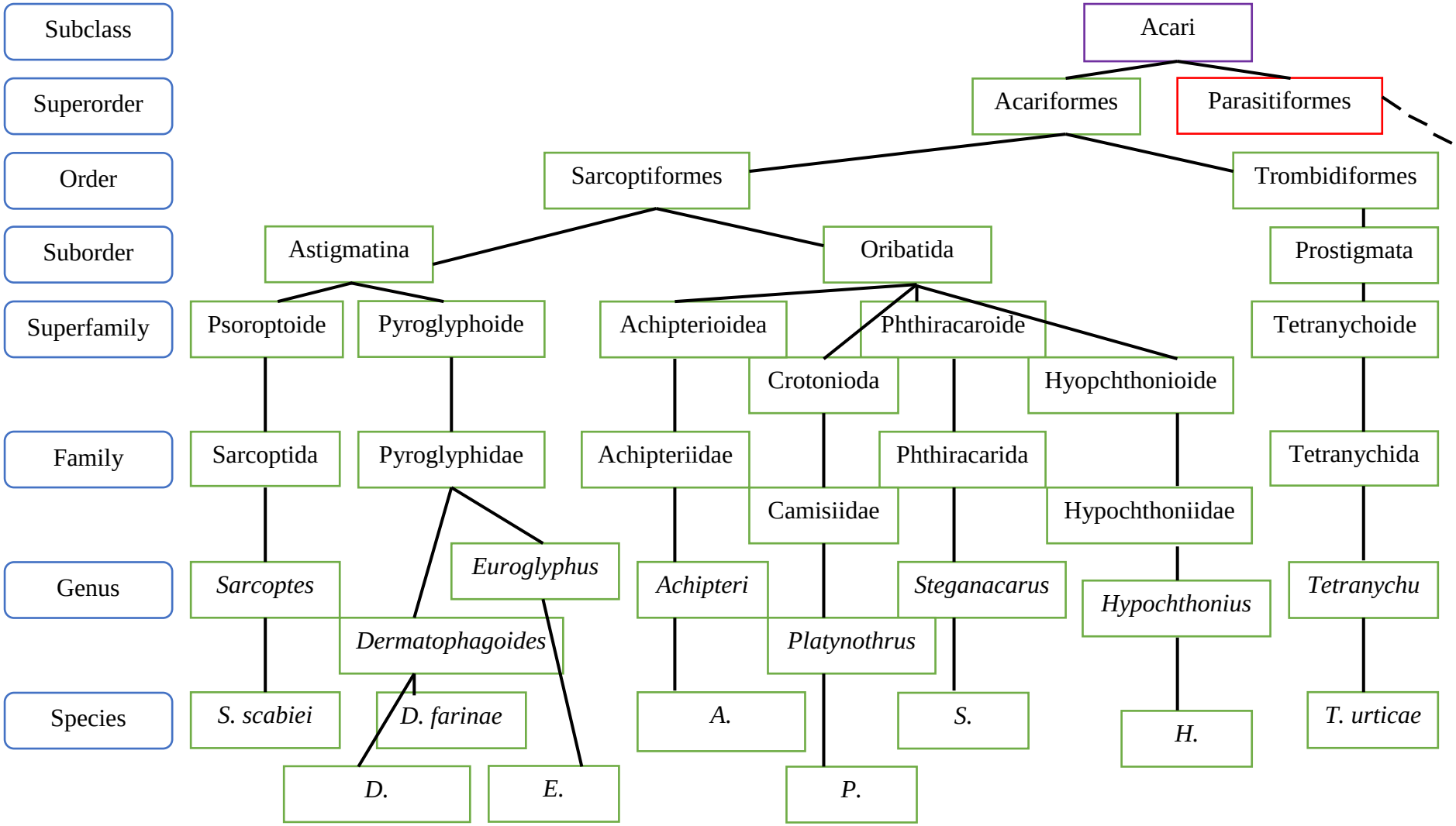


Figure 1.1. Taxonomic tree with published genomes on GenBank (2017) of the nine species of Acariformes (Krantz & Walter, 2009, Proctor, 1998). This includes the true members of HDMs, the Pyroglyphidae, which will be the focus in later Chapters. The estimated divergence between *D. farinae* and *D. pteronyssinus* was noted to be 35 MYA (Dabert et al., 2010).

1.2.3 The Parasitiformes:

In contrast, the Parasitiformes were likely predators in the surface of the littoral zone (Krantz & Walter, 2009). The Parasitiformes include the families Mesostigmata, Holothyrida, and Ixodida (ticks) (Hughes, 1976). The fossil record does not date as far back for the Parasitiformes (Krantz & Walter, 2009), and according to TimeTree (Kumar et al., 2017) these two orders diverged an estimated 494 MYA (Jeyaprakash & Hoy, 2009, Dabert et al., 2010). Figure 1.2 illustrates the taxonomic tree of members of the Parasitiformes with published genomes on NCBI GenBank (2017). Kingdom (Animalia), Phylum (Arthropoda), and Class (Arachnida) were not included in either figure. Additionally, unlike the Acariformes, there are no suborders in the Parasitiformes.

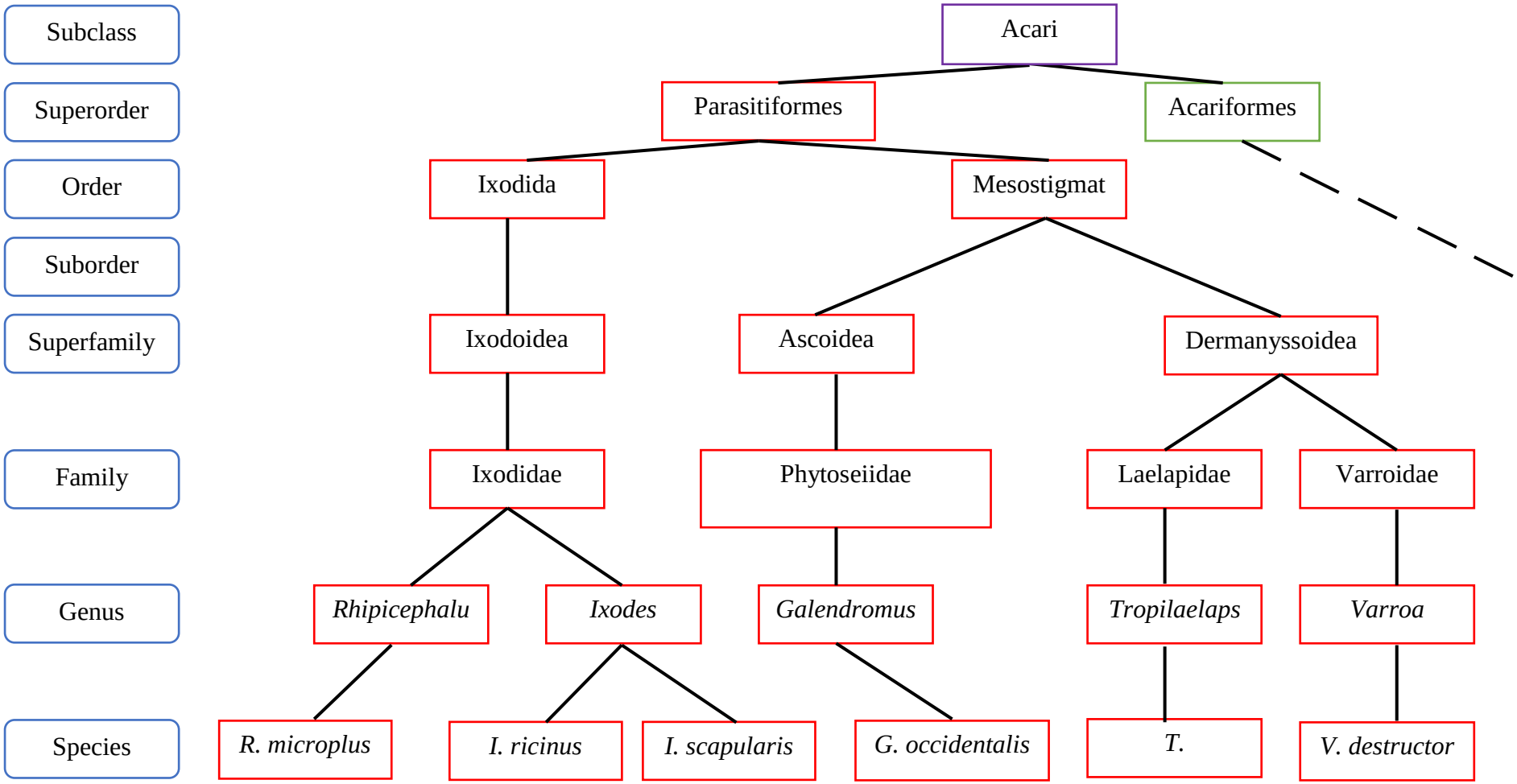


Figure 1.2. Taxonomic tree with published genomes on GenBank (2017) of six species of Parisitiformes (Krantz & Walter, 2009, Walter, 1996). This group includes the ticks, and less common inhabitants of house dust, therefore there is less focus on these species in future Chapters.

1.2.4 Published mite genomes:

There are 15 Acari genomes (mites and ticks) which have been sequenced and assembled on NCBI GenBank (2017). Further information for sequences is provided in Table 1.1, and each species is briefly described below.

The two-spotted spider mite, *Tetranychus urticae* will be discussed later in further detail, as it is the most economically important species of spider mites worldwide (Krantz & Walter, 2009). *Galendromus occidentalis*, known as the western predatory mite, is commonly collected from spider mites. Nomenclature for this species is inconsistent, and may also be listed as a species of *Typhlodromus* or *Metaseiulus*. *Sarcoptes scabiei* (human scabies mite) is a burrowing ectoparasite found underneath the skin surface in small numbers (fewer than 100 mites per host). In susceptible individuals, red patches and intense itching occurs at the site of mite entry. *S. scabiei* can be transmitted by humans to domesticated animals, which can then be transmitted by direct contact to wild animals (Krantz & Walter, 2009).

Achipteria coleoptrata is particularly common in European forest soils, with other members of the species common in temperate soil, litter, mosses and liverworts (Krantz & Walter, 2009). *Hypochthonius rufulus* is also distributed in forests and peatlands in the Nearctic and Palearctic regions (Marshall et al., 1987). It is acidophilous (VanStraalen & Verhoef, 1997), intolerant of heat and drought extremes (Siepel, 1996), and feeds on fungi (Maraun et al., 1998). *Platynothrus peltifer* is an asexual species (Heethoff et al., 2007) which inhabits forest soil and litter, mosses, peatlands, various freshwater habitats, and benthic habitats (Schatz & Gerecke, 1996). It is relatively heat and drought tolerant (Siepel, 1996), and has been widely used in ecotoxicological studies (Krantz & Walter, 2009). *Steganacarus magnus* is a decomposer, particularly in coniferous forests, and contributes to respiratory metabolism in oribatid

communities (Luxton, 1981). It is also drought tolerant and able to survive heat extremes (Siepel, 1996).

Tropilaelaps mercedesae is an ectoparasite of honey bees (*Apis* spp.) (Forsgren et al., 2009). Similarly, *Varroa destructor* is collected from the nests of social insects, where it acts as a hematophagous ectoparasite, and is considered to pose a threat to international beekeeping (Krantz & Walter, 2009). The overabundance of mites on these bees results in reduced weight, along with abnormal body and wing development (DeJong et al., 1982).

There are nearly 700 species in the Ixodidae (hard ticks), most of which are invertebrate pests transmitting diseases with little specificity for a particular host (Krantz & Walter, 2009). The genus *Ixodes* parasitize burrow / den-inhabiting mammals, with two common species *I. ricinus* and *I. scapularis* acting as vectors for the spirochete that transmits Lyme disease in North America, and western Europe, respectively (Krantz & Walter, 2009). In addition, *I. scapularis* also carries the viruses which cause louping-ill, Crimean-Congo haemorrhagic fever, tick-borne encephalitis, and Q fever (Keirans et al., 1999). Similarly, *Rhipicephalus microplus* is a large subgenus of highly economically important ticks which assist in the transmission of cattle fever (Krantz & Walter, 2009).

Over time, *D. farinae*, *D. pteronyssinus*, and *E. maynei* have adapted to be the dominant house dust mites in many areas worldwide, as well as acting as a primary allergen source through their shed exoskeleton and faeces (Krantz & Walter, 2009). These mites, as true species of HDMs, will be discussed in later Chapters.

Table 1.1. Description of 15 Acari genomes (mite and ticks) which have been assembled on NCBI GenBank. *D. farinae* will be the focus of later research in this dissertation. Along with *D. pteronyssinus* and *E. maynei* it is one of the main species of HDM collected worldwide.

Species	**Order Classification**	**GenBank Accession**	**Submitter**	**Date**
Sarcoptes scabiei	Acariformes (Sarcoptiformes: Astigmatina)	GCA_000828355	Wright State University	10/02/2015
Dermatophagoides farinae	Acariformes (Sarcoptiformes: Astigmatina)	GCA_000767015	The Chinese University of Hong Kong	15/10/2014
		GCA_002085665	University of Southern Mississippi	11/04/2017
Dermatophagoides pteronyssinus	Acariformes (Sarcoptiformes: Astigmatina)	GCA_001901225	Maynooth University	21/06/2017
Euroglyphus maynei	Acariformes (Sarcoptiformes: Astigmatina)	GCA_002135145	Wright State University	12/05/2017
Achipteria coleoptrata	Acariformes (Sarcoptiformes: Oribatida)	GCA_000988765	University of Lausanne	06/05/2015
Hypochthonius rufulus	Acariformes (Sarcoptiformes: Oribatida)	GCA_000988845	University of Lausanne	06/05/2015
Platynothrus peltifer	Acariformes (Sarcoptiformes: Oribatida)	GCA_000988905	University of Lausanne	06/05/2015
Steganacarus magnus	Acariformes (Sarcoptiformes: Oribatida)	GCA_000988885	University of Lausanne	06/05/2015
Tetranychus urticae	Acariformes (Trombidiformes: Prostigmata)	GCA_000239435	DOE Joint Genome Institute	20/12/2011
Ixodes ricinus	Parasitiformes (Ixodida)	GCA_000973045	Luxembourg Institute of Health	24/08/2016
Ixodes scapularis	Parasitiformes (Ixodida)	GCA_000208615	Ixodes scapularis Genome Project	18/04/2008
Rhipicephalus microplus	Parasitiformes (Ixodida)	GCA_000181235	USDA-ARS	08/07/2012
		GCA_002176555	Centre for Comparative Genomics	08/06/2017
Galendromus occidentalis	Parasitiformes (Mesostigmata)	GCA_000255335	Baylor College of Medicine	27/03/2012
Tropilaelaps mercedesae	Parasitiformes (Mesostigmata)	GCA_002081605	Xi'an Jiaotong-Liverpool University	06/04/2017
Varroa destructor	Parasitiformes (Mesostigmata)	GCA_000181155	Varroa Genome Sequencing Consortium	09/05/2017

1.3 Relevance of Mites

1.3.1 Medical importance:

There has been a lot of research into HDM fauna due to their adverse effect on human health, as associations can cause chronic diseases including asthma, rhinitis, atopic dermatitis, and conjunctivitis (Colloff et al., 1992). Biochemical composition, sequence homology, and molecular weight has assisted with classifying groups of mites (Arlian & Platts-Mills, 2001). The development of expressed sequence tags (ESTs) for common HDM species by identification of isoallergens (cDNA clones that have slightly differing amino acid sequences from known allergens) has assisted with identifying mite allergens (Angus et al., 2004).

There is no consensus between microenvironments in the home and factors contributing to the mite allergen concentrations. For example, in the city of Chiang Mai in Thailand, mattresses had higher levels of allergen dust than living room floors (Trakultivakorn & Krudtong, 2004). Mattress age, family size, dampness, and mold growth had no effect on allergen concentration, however the type of mattress and presence of a rug in the living room were associated with a concentration increase (Trakultivakorn & Krudtong, 2004). In comparison, analysis of atopic sensitization of children in Stockholm, Sweden, showed a correlation between mite sensitization and house dampness (Nordvall et al., 1988). Also, mite allergens tend to differ between geographic locations due to favoured climatic conditions of mites (Ferrándiz et al., 1996). In Cuba, positive skin reactions are particularly prevalent with *B. tropicalis* and *Dermatophagoides siboney*, with lower levels of sensitization to other common species of HDM which are less less inclined to thrive in such hot conditions (Ferrándiz et al., 1996).

Techniques to eradicate mite allergens within the home involve diminishing the live mite populations, as well as decreasing human exposure to mites and their allergens (Arlian & Platts-Mills, 2001). This involves reducing indoor humidity, using mattress and pillow encasements, conducting weekly washing of bedding materials using hot water (55^{o}C or higher), replacing carpets, curtains and upholstery, frequent vacuuming of carpets, and freezing soft toys (Arlian & Platts-Mills, 2001).

1.3.2 Economic importance:

Many insects and arthropods, including certain species of mites, are crop pests with economic importance. The increase in world food supplies caused by the "green revolution" comes at an ecological, environmental, and socioeconomic cost (Dhaliwal et al., 2010). The improved use of dwarf varieties of crops, as well as increased agrochemicals and irrigation have contributed to the rise of minor crop pests to the stage where they are now major pests. However there is limited quantitative data encompassing the wide variety of pests, which is detrimental to crop protection aims (Dhaliwal et al., 2010).

Phytophagous mites, particularly *Tetranychidae* (spider mites), *Tenuipalpidae* (false spider mites), *Tarsonemidae* (tarsonemid mites), and *Eriophyidae* (gall and rust mites) are a major threat to the production of food, feed and fibre (Van Leeuwen et al., 2010a, Van Leeuwen et al., 2010b). Mites attack by direct feeding, or transmission of plant pathogens and viruses to host plants representing major crops such as vegetables, fruits, corn, soybeans, and cotton (Van Leeuwen et al., 2010a, Van Leeuwen et al., 2010b). As an example, the green mite *Mononychellus tanajoa* and the two-spotted spider mite *T. urticae* have both been reported on the plant Cassava (*Manihot esculenta*) (Bellotti & Vanschoonhoven, 1978). Cassava is a plant cultivated in developing countries which acts as a major energy source of 300-500 million

people. Due to the amount of damage they cause through necroses in stems, leaves, and possible plant death, these mites can decrease crop yield by 46% in Uganda (Nyiira, 1975), 15-20% in Venezuela, and 20-53% in Colombia (Bellotti & Vanschoonhoven, 1978).

In addition, the spider mites are able to develop resistance to modern insecticide (Whalon et al., 2014). To contradict some of these issues, members of the predatory mite family Laelaptidae, can be introduced to control the phytophagous mites which are depleting crop supplies (Evans, 1952). A 2008 review on phytophagous mite data illustrated that almost 80% of acaricides are spent on spider mite control (€400 million) (Van Leeuwen et al., 2015). Three-quarters of these acaricides are sprayed on fruits and vegetables. Acaricides are continuously being developed for specific pests with new modes of action and different chemistries to try to circumvent resistance to insecticides (Van Leeuwen et al., 2015).

1.3.3 Forensic importance:

Various arthropods, including mites, have previously been used by forensic entomologists to estimate post-mortem interval (Goff, 1989). The first documented case of mites being used in this manner was the examination of a mummified body in 1878 (Perotti, 2009). Insects such as blowflies, fleshflies, and beetles are commonly used in forensic investigations, but the absence of these insects poses a grave problem for the forensic entomologist (Perotti et al., 2009). Due to the small size of mites, they are present in a variety of microenvironments within a home (Amendt et al., 2010), and their size and light weight contribute to them unknowingly being transferred to various locations by humans, animals and corpses (Perotti & Braig, 2009). As such, DNA from mites could potentially be used as trace evidence, by performing population genetic analysis to link a mite (and person) to their original location (Perotti & Braig, 2009).

1.4 Various Genetic Systems in Mites

1.4.1 Diploidy:

There is a wide range of mating systems in mites, which makes them useful for studying genetic systems. Detailed reviews of methods of different modes of reproduction in various members of the Acari can be found in Oliver (1977) and Oliver (1971). Table 1.2 provides a list of four different mating systems and some mite species and families which reproduce using this system.

As previously outlined, Parasitiformes and Acariformes are the two distinct taxa within the Acari (Krantz & Walter, 2009, Wrensch et al., 1994). In both orders, the ancestral mode of reproduction was diploidy, without separate sex chromosomes (Wrensch et al., 1994). This ancestral method was retained in certain members of the Acari, including members of the Ixodida (ticks), Mesostigmata, Oribatida, Astigmata, and Parasitengona. However, some of these species also reproduce by other methods to become haplodiploid (females are diploid, males are haploid). In many instances, distinct sex chromosomes have evolved with mostly XO or XY males (Wrensch et al., 1994). Diploidy reproduction is exemplified by similar levels of heterozygosity in males and females (Palmer & Norton, 1992).

1.4.2 Thelytoky:

There are two other main methods of reproduction for mites: thelytoky and arrhenotoky. Thelytokous parthenogenesis results in unfertilized eggs developing into diploid females (Goudie & Oldroyd, 2014, Pearcy et al., 2004). This mating system is exemplified by reduced genetic diversity and skewed sex ratios in mite species (Palmer & Norton, 1992). Thelytokous mites have varying levels of genetic variation between populations, and the genomes in a

population may have been inherited as a unit. This may have been caused by only one incident of thelytoky in the past, or differences in the length of time since becoming asexual (Palmer & Norton, 1992, Helle et al., 1980, Welbourn et al., 2003). Thelytoky can arise through genetic means, or is endosymbiont induced, often through *Cardinium* or *Wolbachia* bacteria (Groot & Breeuwer, 2006, Cruickshank & Thomas, 1999). Thelytoky is present in all suborders of mites, apart from Notostigmata and Tetrastigmata (due to limited data), but it is not considered the major reproductive mechanism in mite families (Oliver, 1971). In approximately 300 species of the genus *Brevipalpus*, both thelytokous and arrhenotokous mechanisms are present (Weeks et al., 2001).

1.4.3 Arrhenotoky:

In comparison, arrhenotokous reproduction is a form of parthenogenesis in which fertilized eggs develop into diploid females, whereas unfertilized eggs develop into haploid males (Goudie & Oldroyd, 2014). The term arrhenotoky has been used interchangeably in literature with haplodiploidy or pseudo-arrhenotoky, however these are distinct occurrences and can occur independently (Cruickshank & Thomas, 1999). This is a common method of reproduction in the Acari (Helle et al., 1978).

However, there are no reported instances of arrhenotoky in any species of tick or oribatid mites (Oliver, 1971). In the oribatid mites, it has been theorised that both sexual and parthenogenetic species undergo a meiotic process (Wrensch et al., 1994). Parthenogenic lineages likely arose through occasional sexual lineages, and did not diversify (Maraun et al., 2003). The larger differences in age between four closely related parthenogenetic species compared to the age between four closely related sexual species provides support that some parthenogenetic oribatid

mite species may radiate slower than the sexual species, and are “ancient asexuals” (Maraun et al., 2003).

1.4.4 Pseudo-arrhenotoky:

Pseudo-arrhenotoky results from diploid males expelling the paternal genome or retaining it in somatic cells, which leads to haplodiploidy in the individuals (Cruickshank & Thomas, 1999, Wrensch et al., 1994). As in arrhenotokous reproduction, females have twice as many copies of genes as males (Hedrick & Parker, 1997). This has an influence on allelic frequencies, mutation rates, and recombination rates (Hedrick & Parker, 1997).

Haplodiploidy will be discussed in greater detail in Chapter 6. In general, haplodiploids are not as affected by inbreeding depression (reduction in offspring fitness from inbred parents, compared to offspring from unrelated parents) as diploid species (Tien et al., 2015). In outbred populations which suddenly experience inbreeding, homozygosity is increased, which leads to unfavourable genetic material being expressed (Henter, 2003). Unfavourable recessive deleterious mutations are masked in heterozygous females, but will be immediately exposed and removed in haploid males (Werren, 1993). Consequently, it is likely that traits which are expressed and subject to selection in males have a reduction in inbreeding depression. Additionally, the lower ploidy level in haplodiploids may also result in an overall lower “effective” mutation rate and reduced unfavourable genetic material (genetic load), even in traits which are only expressed in females (Werren, 1993).

Table 1.2. Different mating systems identified in mites, and some species which are known to reproduce in this manner.

Mating System	**Mite Species/ Family**	**Additional Information**	**Reference**
Diploidy	*Heminothrus gibba*	Sexual	(Oliver, 1977)
	Acarids	XO sex determination system with the exception of *Rhizoglyphus echinopus* (no sex chromosomes)	(Oliver, 1977)
Thelytoky	Desmonomata		(Palmer & Norton, 1992)
	Eylais rimosa and *Eylais setosa*	Two chromosomes	(Moss et al., 1968)
	Tetranyccopsis horridus	2n=4	(Helle & Bolland, 1967)
	Lorryia formosa		(Hernandes et al 2006)
	Brevipalpus californicus and *Brevipalpus obovatus*		(Helle et al., 1980)
	Brevipalpus phoenicis	Due to endosymbiotic bacterium feminizing haploid genetic males, both male and female parthenogens are haploid	(Weeks et al., 2001)
Arrhenotoky	Mesostigmata, Macrochelidae, Dermanyssidae, Macronyssidae and Phytoseiidae		
	Astigmata, Prostigmata, Cheyletoidea and Tetranychoidea		
	Harpyrhynchus novoplumaris	n=2; 2n=4	
	Ornithonyssus bacoti and *Ophionyssus natricis*	n=8; 2n=16 and n=9; 2n=18	
	Rattus assimilis and *Anoetus laboratorium*	n=7; 2n=14 and n=4; 2n=8	
	12 species of Tetranychidae (exception *Tetranycopsis horridus* which is a diploid species which undergoes thelytokous parthenogenesis)	Male n=2-7, female 2n=double the corresponding haploid male	
Haplodiploidy	Otopheidomenidae (including *Dicrocheles phalaenodectes* and *Hemipteroseius wormersleyi*)		(Treat, 1965)
	Phytoseiidae (including *Amblyseius cucmeris, Phytoseiulus persimilis, Typhlodromus occidentalis* and *Typhlodromus pyri*)		(Treat, 1965, Hoy, 1979)

1.5 Population Genetic Structure in Mites:

1.5.1 Fragmented habitats:

As genes are exchanged between populations, allele frequencies are homogenised and geographical isolation can result in closer populations being more genetically similar than distant populations (Balloux & Goudet, 2002). This results in a population genetic structure. Small isolated populations are particularly affected by genetic drift if deleterious mutations become fixed in the population (Balloux & Goudet, 2002). Mite diversity and population structure may be affected by habitat fragmentation (Gibbs & Stanton, 2001). For example, phoretic mites in temperature forests attach to carrion beetles (Silphidae) and are transported to decomposing carcasses, where they disembark and reproduce. Although the mite loads on beetles in both fragmented and contiguous forests were comparable, mite variance increased in fragmented sites. The reasoning behind these differences remain unknown, however it was not due to changes in the composition of beetle communities (Gibbs & Stanton, 2001).

Additionally, the presence of fragmented habitats may lead to patchy food distribution, where the presence of food in discontinuous patches would prevent mite populations at each source from interbreeding with mites from other populations. In species such as copepods (Arthropoda: Crustacea), movement between isolated food sources is influenced by the length of time they can survive without food with no significant physiological affect (Dagg, 1977). Although a patch is theoretically noted as a well-defined area where food is uniformly distributed with no food between patches, there is a possibility of non-patchy habitats (Arditi & Dacorogna, 1988). In these cases, animals could travel and feed whist moving across their environment (Arditi & Dacorogna, 1988). Therefore, mite populations could either move from

isolated food patches allowing the populations to mix, or the mites could remain in isolated microenvironments, creating geographically isolated and distinct populations.

1.5.2 *Tetraynchus urticae* population structure:

Microsatellites have been used to examine *T. urticae* population genetic structure. The population structure of *T. urticae* may be influenced by host plant as well as geographical distances between populations (Sauné et al., 2015). Multiple populations of this species illustrate heterozygote deficiency leading to deviations from Hardy-Weinberg Equilibrium (HWE) (Carbonnelle et al., 2007, Sun et al., 2012, Sauné et al., 2015). Mites tend to have a higher heterozygote deficiency due to distribution in isolated patches at the beginning of the season, compared to later in the season following complete panmixia (Carbonnelle et al., 2007). Inbreeding may influence genetic variation (Carbonnelle et al., 2007), and low genetic diversity within each population of *T. urticae* is likely caused by the effects of genetic drift over time (Sun et al., 2012).

In China, *T. urticae* populations of both the native red mites and invasive green mites displayed deviations from HWE (Sun et al., 2012). Genetic diversity was higher in the red mites, potentially due to a founder effect reducing genetic diversity in the green mites. Additionally, diversity was inversely correlated with latitude, possibly due to a higher mutation rate in southern populations (Sun et al., 2012). Compared to other species, *T. urticae* has low level microsatellite polymorphism, potentially because it is an invasive species which underwent a bottleneck during invasion (Zhang et al., 2016).

As previously stated, the next two Chapters will focus on the worldwide distribution of HDMs, through the creation of a database from published resources.

Chapter 2: Compiling a Database of House Dust Mites and Geographic Location

2.1 Introduction

The original definition of house dust mites (HDMs), by Arlian and Platts-Mills (2001) encompassed only mites belonging to the Acarine, and family Pyroglyphidae, however over time this definition has widened to include other families of mites such as the Acaridae, Glycyphagidae, Cheyletidae, and Oribatid (Colloff, 2009). Despite the numerous mites collected from house dust, the majority are likely contaminants introduced into an indoor environment on plants, animals, soil, or humans (van Bronswijk, 1981). The natural habitat of HDMs are locations where they feed on fungi, organic material, and bacteria (Klimov & O'Connor, 2013). Over time, mites have phoretically associated with birds and mammals (Colloff, 2009). Through this relationship, mites could be mechanically transferred by these hosts to increase their diversification, eventually becoming contaminants within the home (Colloff, 2009).

As outlined in the previous chapter, the majority of research into mite fauna has been focused on the medical and economic implications of these species; however, there is not a recent comprehensive report of all indoors HDM fauna worldwide. As such, a database of HDM fauna worldwide based on all accessible published resources was created. From this, species diversity between countries and biogeographical regions were compared to assess the completeness of the database. In addition a variety of factors were examined in countries with high levels of research as applications of the HDM database.

2.2 Materials and Methods

2.2.1 Collection of published research:

Examination of all published journal articles from 1950 to the beginning of 2017 available in the United Kingdom containing relevant information of the global distribution and abundance of HDMs identified 347 publications on HDMs. This involved online searches using Web of Science, Google Scholar, EThOs, ProQuest Dissertations & Theses Global, and Summon2.0. Searches were conducted systematically by decade, and involved the terms: "house dust mite*", "*Dermatophagoides*", "*Euroglyphus*", "*Blomia*", "worldwide", "distribution", and "abundance". The following exclusion terms were also employed to reduce the number of non-relevant publications: "allerg*", "asthma", "rhinitis", and "skin prick test". Subsequently, interlibrary loans, archives in the University of Reading collection, as well as the resources available at the University College of London library were used to acquire publications that were not available online or through the University of Reading journal subscription catalogue. Publications were separated by country, and then biogeographical realm (Figure 2.1) (Olson et al., 2001).

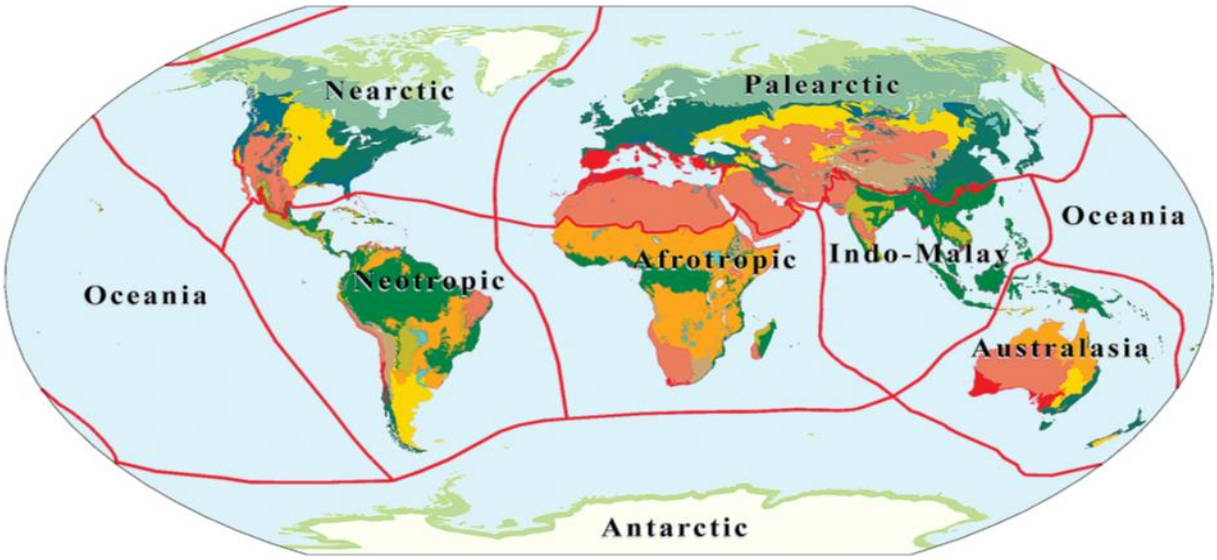


Figure 2.1. Map of the generalised biogeographical realms of the world. HDM fauna was categorised within the Afrotropical, Australasian, Indo-Malay, Nearctic, Neotropical, and Palearctic East and West regions.

2.2.2 Publication parameters:

To consolidate the number of publications included in the review to a manageable level, some parameters were enacted. Only mites which were collected from a location people were living (ie sleeping and eating on a regular basis) were included. This comprised houses, flats, university dormitories, and farmhouses. Studies which examined mite fauna in other indoor locations, including but not limited to, hospitals, schools, day care centres, bakeries, research laboratories, libraries, and offices, were not included. However, mites collected from hospital staff clothing, or other clothing were encompassed in this study. Although it is highly likely that mites would be transferred on people between locations, studies from outdoor or agricultural locations were not included. This involved locations such as passenger trains, hire cars, grocery stores involving stored food, and farming environments / agricultural areas.

Furthermore, this review excluded mites which were collected and identified following an allergic reaction, anaphylactic shock or from human sputum following ingestion. Finally, only mite species which were morphologically identified, rather than DNA identified, were included. As such, publications which identified the mite species by only a positive skin prick test for the allergen, (ie positive reaction for the *Der p* 1 allergen being indicative of *D. pteronyssinus*) were not counted. The rationale for only including morphologically identified mites was that these mites were undeniably present in the house at the time of collection. A positive skin prick test is indicative of an allergic reaction to a particular mite species. While this suggests the presence of that species, I did not believe it was conclusive evidence of that mite in the examined home, as exposure to the allergenic mites could occur in other locations outside of the home.

2.3 Results and Discussion

2.3.1 Database from the literature:

A database of HDM fauna from the literature was published with the University of Reading's Data Repository, the Research Data Archive (http://dx.doi.org/10.17864/1947.145) (Farncombe, 2018). The HDM fauna database was compiled in Microsoft Excel and contains two tables with 347 articles and 531 species. It is important to note that some publications examined multiple countries within the same article. Therefore although 347 publications were collected, there are a total of 363 study numbers in the database, as the mite fauna for each country was individually recorded.

Each species name is linked by ID number to the publication source. All taxon names were analysed to account for spelling errors and language barriers, however misidentification may still have inadvertently occurred. For all mite fauna, if the publication was listed with "sp." or "spp.", it was changed to the genus name for consistency and to consolidate the number of species. Unidentified samples are listed as "other". Geographical information is also recorded, both by biogeographical region and country level. Comments regarding the study, including year of study (if given), housing, socioeconomic factors, presence of pets, and state / province, were noted.

Figure 2.2 illustrates the countries where studies of HDM fauna have been conducted, with each biogeographical region represented by a different colour. Some judgement calls were made which resulted in slight variations from Figure 2.1 (ie biogeographical realm of Algeria), and China is an intermediate colour due to being classified as part of both the Indo-Malay and Palearctic East regions.

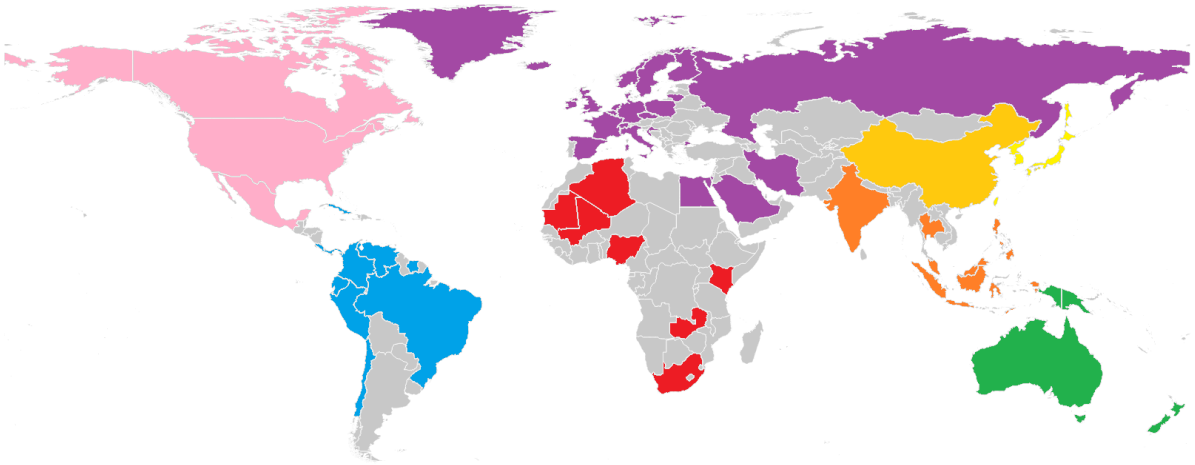


Figure 2.2. Coloured-coded illustration of where research into HDM fauna has occurred. Coloured biogeographical regions as follows: Afrotropical; Australasian; Indo-Malay; Nearctic; Neotropical; Palearctic East; and Palearctic West.

Table 2.2, provided in Appendix 1, illustrates the biogeographical region, country of collection, and reference(s) for all publications. The number of times each species was identified out of 363 publications was calculated. Figure 2.3 illustrates the 37 most common HDM species, collected from a minimum of 5% of publications. The most frequently collected species worldwide were *D. pteronyssinus* (298 – 82.1%), followed by *D. fariane* (235 – 64.7%), *E. maynei* (155 – 42.7%), and *Tyrophagus putrescentiae* (132 – 24.9%).

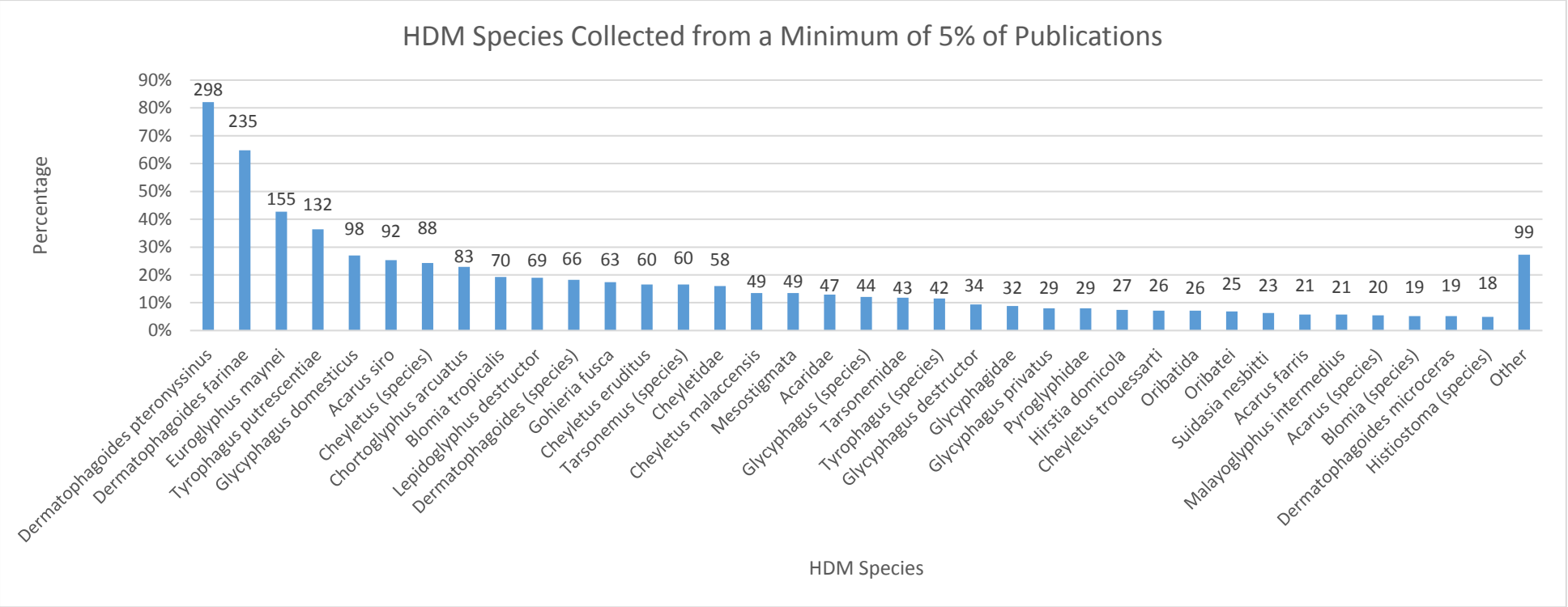


Figure 2.3. Number of times HDMs were collected (out of 363 studies) noted on the top of the bars, along with the percentage of each species out of all publications. Information is only provided for species identified in at least 5% of publications.

The other 494 species not included in Figure 2.3 were found at a frequency of less than 5%, with 368 of those species collected at a frequency of less than 1%. The natural habitat for many of these rarely identified species is not in homes or house dust (van Bronswijk, 1981). As such, these species would not be considered true HDMs, and have instead been inadvertently transferred indoors on plants, animals, soil, or humans (van Bronswijk, 1981). For example, members of the suborder Oribatida inhabit the soil-litter system, and are frequently collected from temperate forests (Krantz & Walter, 2009). Mites from the order Mesostigmata have highly varied habitats, including soil, litter, rotting wood, compost, manure, carrion, nests, and house dust. In addition, the order Trombidiformes has 38 recognised superfamilies, which are highly diverse and occupy a wide variety of habitats (Krantz & Walter, 2009). Therefore, many mites are well known to occupy natural habitats which do not readily include a home environment, indicating that they are contaminants within the house.

2.3.2 Assessing completeness of the database:

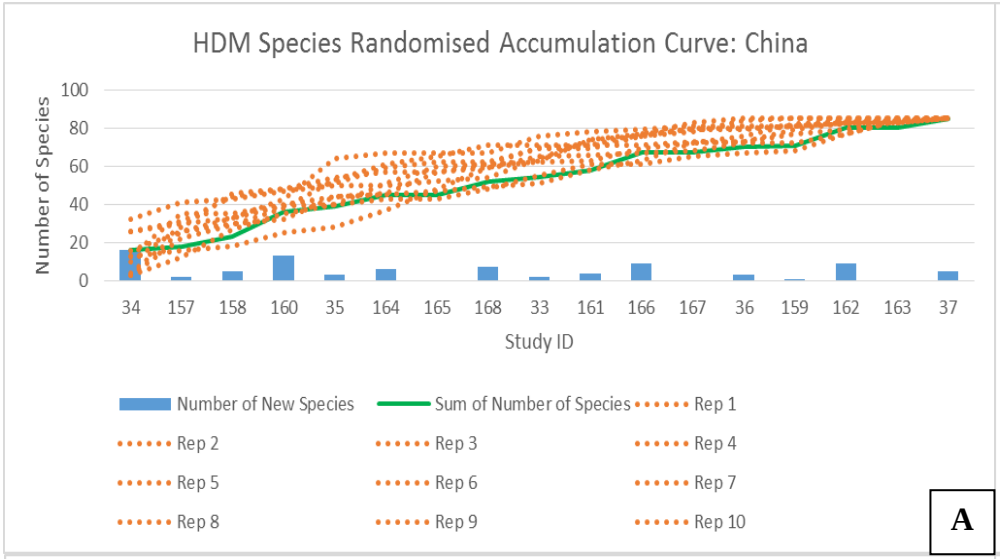
Randomised accumulation curves were created to determine if there is a minimum number of publications, above which no new species of HDMs will be collected from a country. If a country was sampled at regular intervals, the total number of species would slowly accumulate and plateau over time as it starts to approach the true number of species for that area. However, if there was a very thorough survey conducted at the beginning, followed by less thorough surveys from that point onwards, then the curve may falsely appear to plateau. In this instance, there would still be many species to find even if the curves indicate all species have been collected. Taking replicates of randomised accumulation curves should help distinguish between these two situations. If all the randomised curves plateau (including the original and all replicates), then it is likely the true species count for that country is being approached. Initially, there will be a high number of species identified, and then as either the area of the

study decreases or time progresses, the accumulation of the species will decline as only rarer species are collected and an asymptote is reached (Ugland et al., 2003). As the order in which new species are added to a species-accumulation curve will have an effect on the curve, the data was randomised after the initial examination, to prevent the bias which would have occurred had the information been organised chronologically by year (Ugland et al., 2003). The curves of the 13 countries with the highest level of research (greater than 10 publications) were created as follows:

1) The database matrix illustrated the presence / absence of each species from every study. Studies were arranged chronologically by increasing year of publication.
2) It was determined when the species was first identified across all studies ("new species") in the examined country.
3) The number of "new species" were summed with the addition of each study.
4) A graph illustrated by study in order of increasing year the number of "new species" (blue bars). The number of "new species" were summed over each study (solid green line).
5) Then the studies were randomised (no longer categorised by year), by assigning a number from 1 to x, where x was the total number of publications from that country.
6) Excel arbitrarily generated a number between 0 and 1 which was randomly ranked. These numbers corresponded to the study ID numbers from the database and enabled the studies to be reordered without prejudice.
7) The matrix information illustrating the presence / absence of each species per study was rearranged based on the newly randomised study order.
8) As before, it was determined when the species was first identified across all studies ("new species") in the examined country.

9) This provided the number of "new species" per study, and again, the number of "new species" were summed with the addition of each study.
10) 10 iterations were performed, providing 10 randomised replicates of the sum of number of new species.
11) Results were graphed, illustrating only the sum of number of species (dotted orange lines). Study ID is provided along the x-axis. This corresponds to the study ID given in the database, and is shown with increasing publication year.
12) All steps were repeated independently for all 13 countries which had greater than 10 publications.

A total of 13 countries with a minimum of 10 publications were individually examined: Australia, Brazil, China, Denmark, Egypt, India, Japan, Poland, Spain, The Netherlands, Turkey, United Kingdom, and the United States of America, with the results plotted in Figure 2.4. I did not want to examine countries with only a few publications, as I knew they may not be accurate for the number of different species due to the low amount of research. Based on the information available, I decided to use 10 as the threshold value as above this value there was almost an incremental step-wise pattern of number of publications. Below 10, the next closest number of publications was seven and there were multiple countries for each of the values. From these graphs, it should be clear whether the number of studies which have been conducted in each country is enough to have collected all available species of HDM, or whether more research is necessary to identify the total number of HDM species.


HDM Species Randomised Accumulation Curve: China
Number of Species
100
80
60
40
20
0
34 157 158 160 35 164 165 168 33 161 166 167 36 159 162 163 37
Study ID
Number of New Species
Sum of Number of Species
Rep 1
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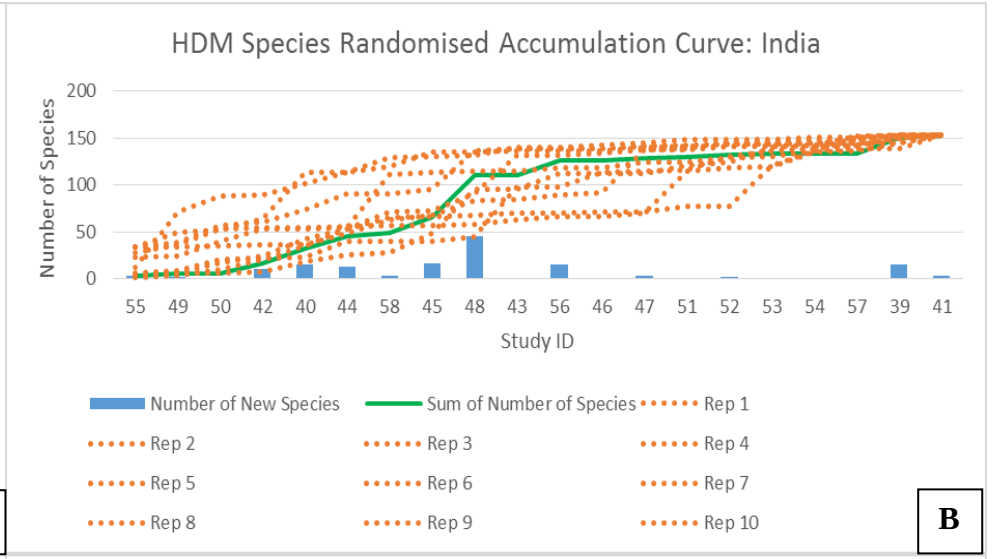
A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Number of Species
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150
100
50
0
55 49 50 42 40 44 58 45 48 43 56 46 47 51 52 53 54 57 39 41
Study ID
Number of New Species
Sum of Number of Species
Rep 1
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Rep 4
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Rep 7
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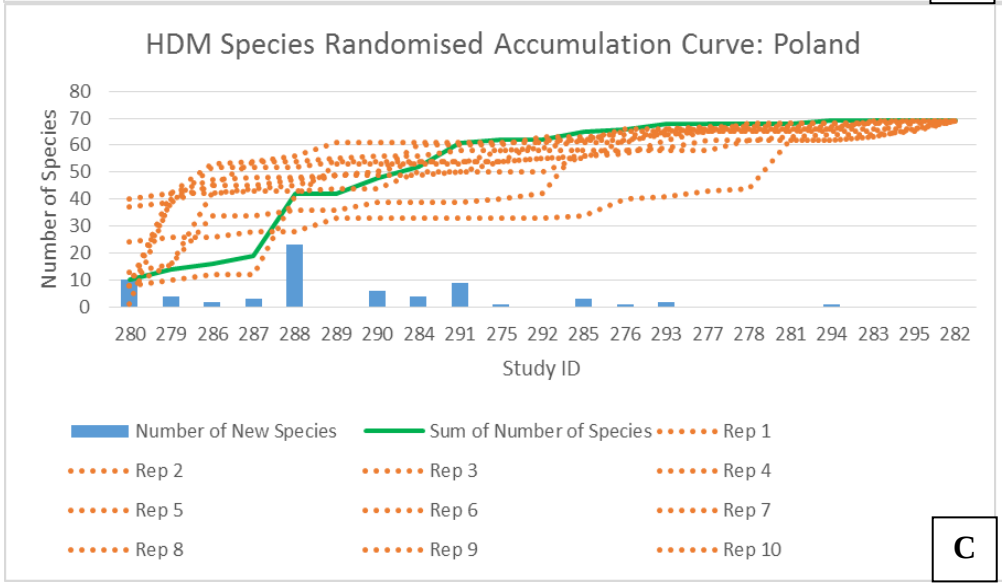
B


HDM Species Randomised Accumulation Curve: Poland
Number of Species
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60
50
40
30
20
10
0
280 279 286 287 288 289 290 284 291 275 292 285 276 293 277 278 281 294 283 295 282
Study ID
Number of New Species
Sum of Number of Species
Rep 1
Rep 2
Rep 3
Rep 4
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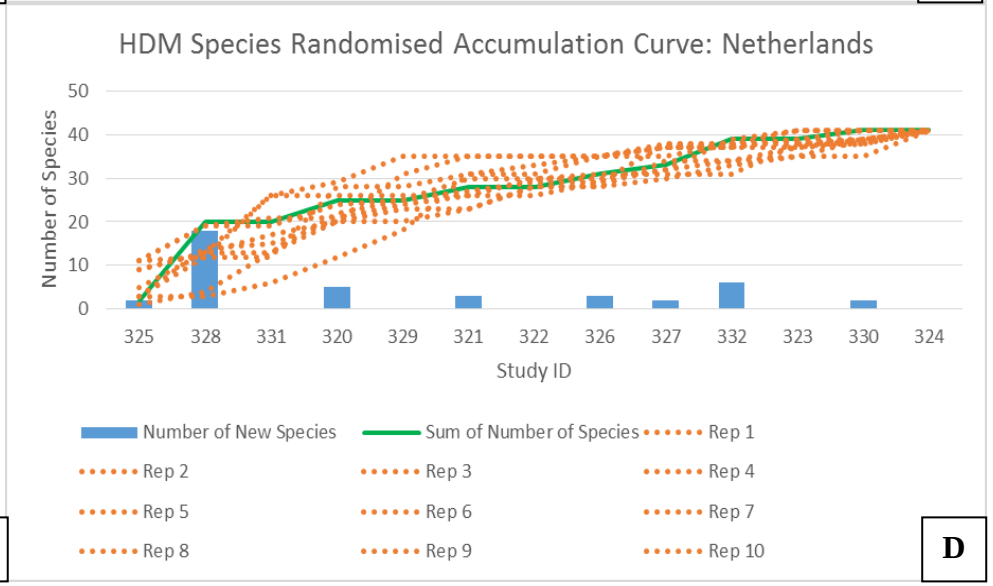
HDM Species Randomised Accumulation Curve: Netherlands
Number of Species
50
40
30
20
10
0
325 328 331 320 329 321 322 326 327 332 323 330 324
Study ID
Number of New Species
Sum of Number of Species
Rep 1
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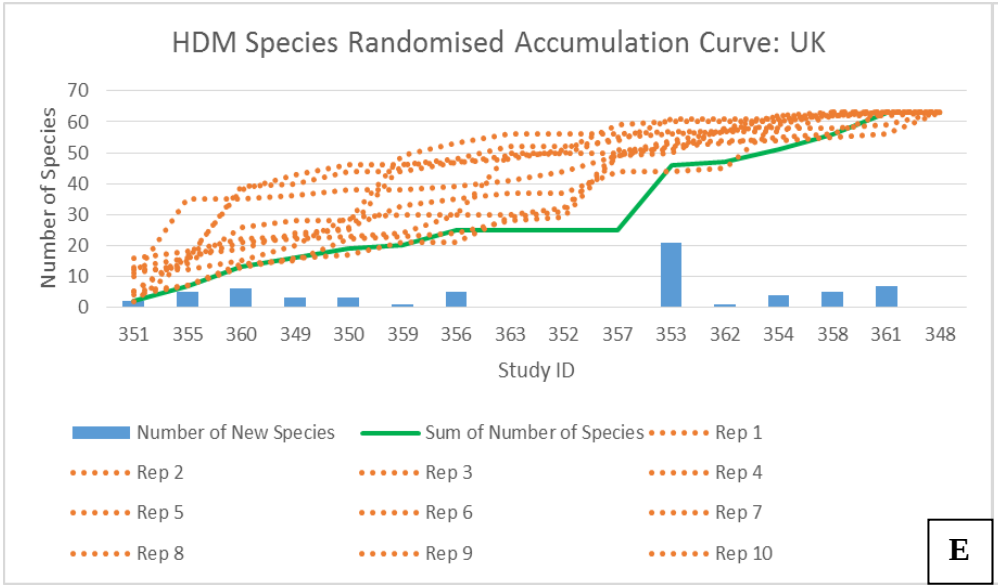
HDM Species Randomised Accumulation Curve: UK
Number of Species
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60
50
40
30
20
10
0
351 355 360 349 350 359 356 363 352 357 353 362 354 358 361 348
Study ID
Number of New Species
Sum of Number of Species
Rep 1
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Rep 4
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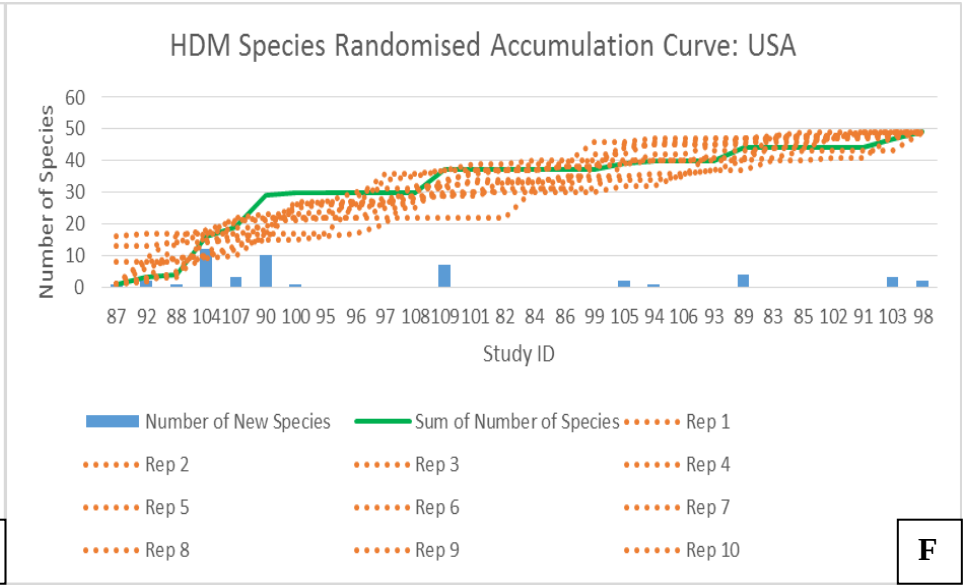
E


HDM Species Randomised Accumulation Curve: USA
Number of Species
60
50
40
30
20
10
0
87 92 88 104 107 90 100 95 96 97 108 109 101 82 84 86 99 105 94 106 93 89 83 85 102 91 103 98
Study ID
Number of New Species
Sum of Number of Species
Rep 1
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Rep 3
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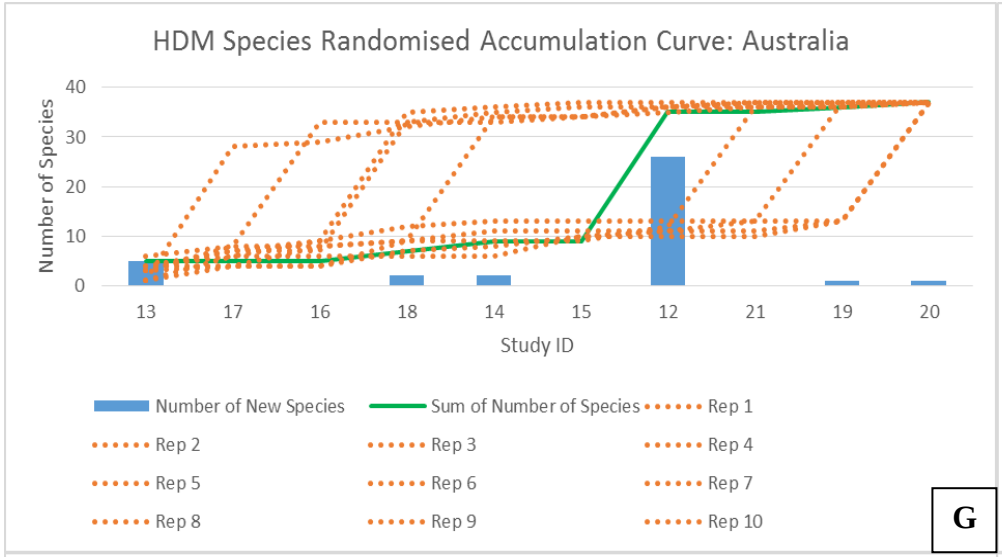
F


HDM Species Randomised Accumulation Curve: Australia
Number of Species
Study ID
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Number of New Species
Sum of Number of Species
Rep 1
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Rep 4
Rep 5
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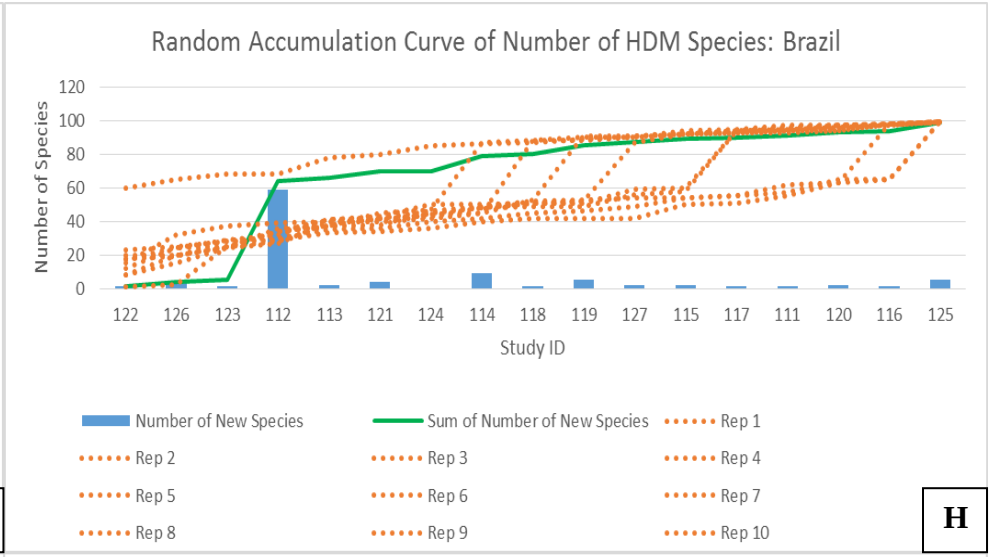
Random Accumulation Curve of Number of HDM Species: Brazil
Number of Species
Study ID
122 126 123 112 113 121 124 114 118 119 127 115 117 111 120 116 125
Number of New Species
Sum of Number of New Species
Rep 1
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Rep 3
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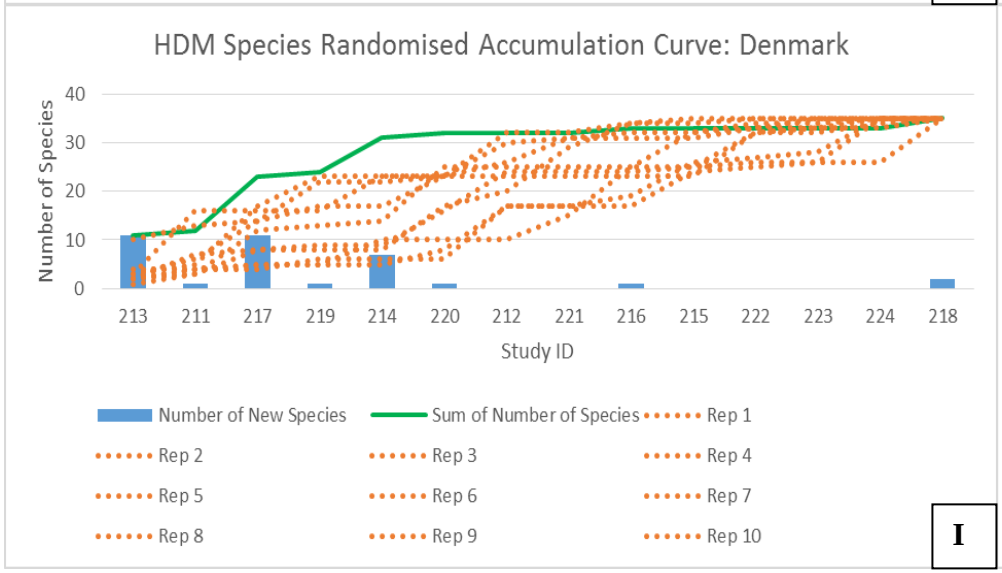
HDM Species Randomised Accumulation Curve: Denmark
Number of Species
Study ID
213 211 217 219 214 220 212 221 216 215 222 223 224 218
Number of New Species
Sum of Number of Species
Rep 1
Rep 2
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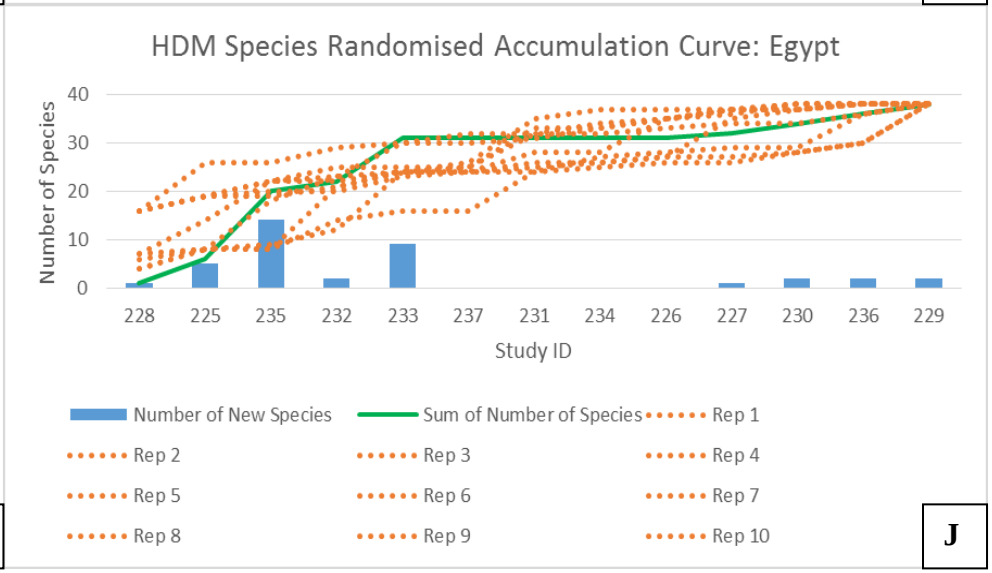
HDM Species Randomised Accumulation Curve: Egypt
Number of Species
Study ID
228 225 235 232 233 237 231 234 226 227 230 236 229
Number of New Species
Sum of Number of Species
Rep 1
Rep 2
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Rep 4
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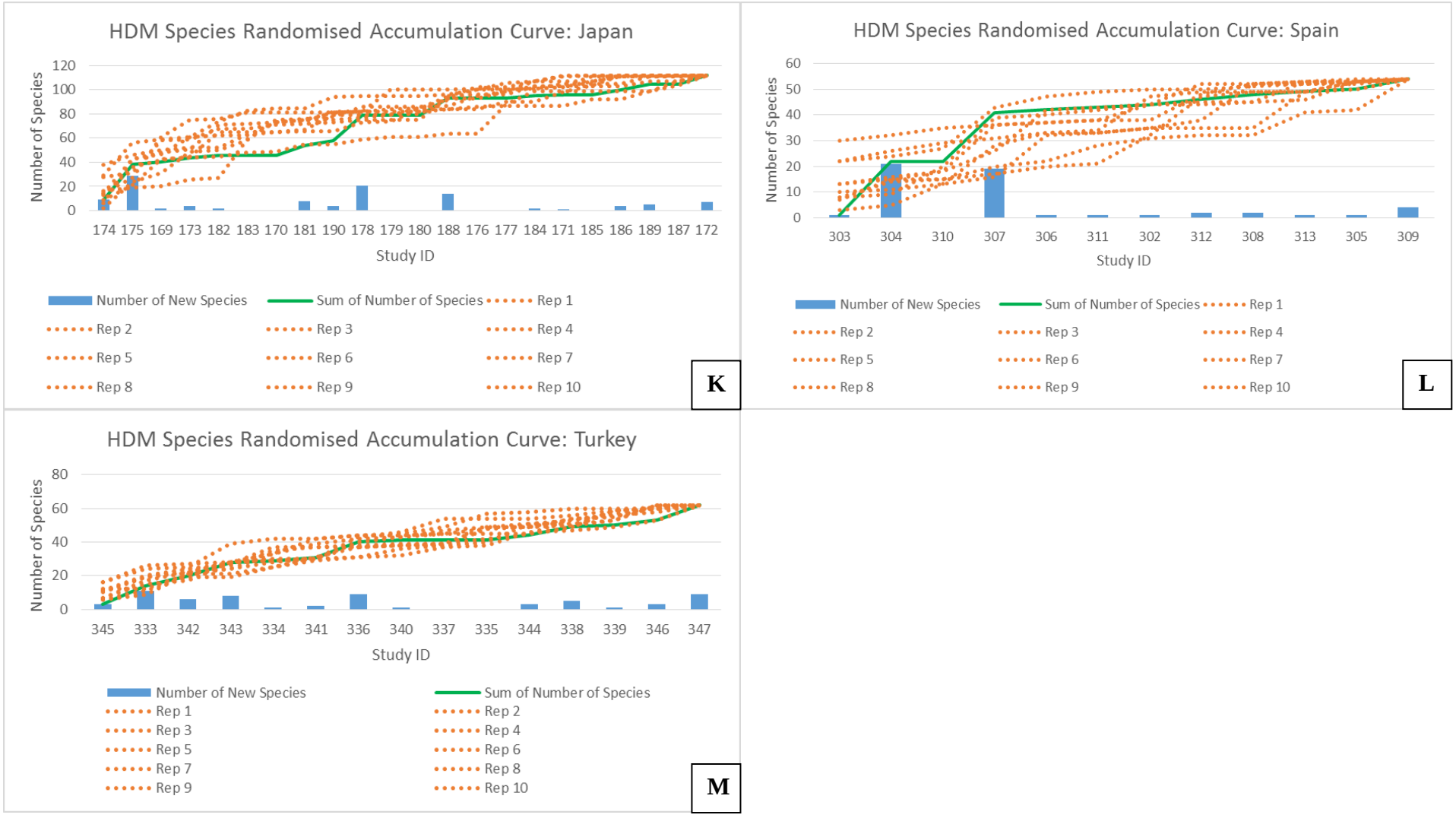


Figure 2.4. Randomised accumulation curves of number of HDM species illustrating the number of new species (blue bar) and the sum of number of new species (solid green line) by increasing year of study publication. In each country, study numbers were then randomised and 10 replicates were taken (dotted orange lines). Figure 2.4a-f indicate a plateau in species numbers, where Figure 2.4g-m do not seem to reach a plateau.

Out of the 13 examined countries, there are six countries that do appear to reach a plateau in the number of species after the original sampling and 10 replicates (Figure 2.4a-f). The curves for China are all increasing at a gradual rate, suggesting that all studies have identified equivalent number of HDM species. A plateau seems to have been reached around 80 species. India seems to be levelling at around 150 species, as is Poland at around 70 species, the Netherlands at around 40 species, the UK around 60 species, and the USA around 50 species. This provides support that all HDM species have been identified in these countries, and further sampling and publications would not identify any additional species.

In comparison, Australia, Brazil, Denmark, Egypt, Japan, Spain, and Turkey do not appear to reach a plateau with regards to the number of new species (Figure 2.4g-m). There is still an increase with the number of new species following the original sampling, as well as the 10 randomised replicates. When the number of species is still rising after all publications, it is indicative that not all HDM species have been collected from these countries. There are some possible explanations for not identifying all HDM fauna. For example, collection methods may have improved over time, and perhaps more recent research looks more intensively into all mite fauna within a home, rather than only searching for one family of mites. As acarologists increase their knowledge of mite fauna, they may be becoming more adept at identifying different species of mites, particularly those which are less common. Increased knowledge into HDM taxonomy means that if mites can be characterised by their species name, instead of by their family name or "unidentified", the number of new species collected from a country would increase. In addition, these countries may simply have not been sampled thoroughly enough, or research may not have encompassed the entire country in different geographical locations.

This does, however, make it difficult to determine a threshold number of publications, above which no new species of HDM would be collected. In countries where minimal research has been conducted (ie one or two studies), it is reasonable to assume that more research is required to identify all HDM fauna. Nevertheless, Figure 2.4g-m indicates that despite a minimum of 10 publications, there are still some unidentified species from seven of 13 countries. Upon examining the six countries which identified all HDM species (Figure 2.4a-f), apart from the Netherlands, the plateau began around 13 publications (China, Poland and the UK). However, in India 16 publications were needed, and the USA needed 19 studies for mite fauna to begin to plateau. This suggests that 10 publications was too low a value to identify all mite fauna within a country, and a minimum of 15 publications may be more accurate to determine HDM fauna. Therefore, there likely remains more HDM fauna to be identified outside of this database.

2.3.3 Limitations of the dataset and how they have been mitigated:

a. Number of studies:

Although a comprehensive database of current HDM fauna worldwide was generated from publications, there are a variety of issues associated with examining mite fauna in this manner. Firstly, there is a large discrepancy in the number of studies conducted within one region or country. There are a variety of factors which may have an influence on the amount of research in a single country, including the level of research funding available. Despite HDMs correlation with allergens, there may be more significant research for funding to go towards in certain countries, negating the need for extensive HDM research. Issues regarding the number of publications has been addressed in Section 2.3.2.

b. External variants:

Consequently, many studies from one country at a variety of locations are necessary to provide an accurate representation of the mite fauna. Certain species of mites are more prevalent at specific temperatures, relative humidity, and altitudes. There are many geographical differences between regions in a single country, for example rural vs. urban, inland vs. coastal, and low vs. high altitude. Additionally, differences exist with types of housing including age of house, material, number of occupants, length of occupation, furnishings, and a variety of socioeconomic factors. As such, HDM fauna can vary between these conditions and therefore a broad number of studies would need to be carried out in each country.

Additionally, there are variations with seaonality, length of sampling time, and whether additional factors such as presence of pets, temperature, relative humidity, and housing conditions were recorded and considered. Some publications may also have only been searching for specific type of mite (ie tarsonemid, storage mites, *Dermatophagoides* spp.). Therefore, even if other mites were present they may have only be listed as "unidentified" or completely excluded from the results. Following from this, there may also be an issue of misidentification. This is particularly relevant with older publications before more accurate keys had been produced, as some species are morphologically quite similar and it is easy to mistake one sister species for another.

c. Collection methods:

Another issue surrounds the inconsistency in collection methods between the individual publications. Different techniques may be used for collecting the mites (ie hoovering, brushing, or shaking) which may affect the total number of mites, as well as increasing the chances of a certain

species not being identified. House dust contains detritus material from clothing, furniture, shed skin, pet dander, and other household items, of which can widely vary between homes creating inconsistencies with mite retrieval (Spieksma & Spieksma-Boezeman, 1967). Most often, a 5g portion of dust is used to isolate mites, however samples containing a higher amount of sand will contain less dust than samples with little or no sand. This will influence the number of mites identified in a sample. Afterwards, the dust is sieved, with the size of sieve influencing collection of all possible mites from the dust. Mites may also be missed by untrained scientists searching through the remaining dust particles after treatment (Spieksma & Spieksma-Boezeman, 1967). However, my analysis focused solely on the presence of a species of mite, rather on the number of each mite species or their density or abundance. This was due to inconsistencies in publications regarding density of mites in g/dust or specific number of mite species. Therefore, issues with collection methods influencing the mite fauna in this database are not of a great concern.

d. Publication bias:

One of the main issues associated with this report is a bias towards English-written publications. The online searches may not have encompassed research into HDM fauna from certain countries, possibly due to the journal the research was published in, or the language it was written. As some of the online searches were conducted using the Latin nomenclature for common HDM species, this problem would have been reduced. Some information or articles may also be overlooked due to poor translation, as often the abstract or summary is given in English but not the reminder of the publication.

This became even more difficult with the overall lack of accessibility of primary research articles citing HDM fauna. Older publications were not readily available in libraries. Additionally, publications were often in lesser-known or lower impact journals that did not have an ongoing subscription. Some research was only listed as personal communication, and often the results of a doctoral thesis was cited, but due to country or language, the published version was not accessible.

In a few instances, a review study of the mite fauna had previously been published for a country. A pertinent example would be when 101 publications were created into a review paper of mite fauna in Brazil (Binotti et al., 2001a). For this report, this work was cited as one publication in the database, unless the primary journal article was available and was then referenced as a separate entity in the database. However, the majority of publications were not available from the UK, and could possibly only be accessed at a Brazilian institute in their native Portuguese. As such, the number of publications from Brazil may be much higher than the database indicates. However, although the number of publications would not be accurate, the different HDM species which were collected from Brazil will be correctly recorded in the database.

2.3.4 Applications of the database:

Applications for the HDM database were examined using RStudio (2015c). A table was created which identified the 13 countries with the highest number of publications and the total number of species collected from these publications. This did not identify whether the species was a "new" species, but was a cumulative count across all publications from each country. A second table identified different factors which I thought may contribute to species richness in a country. These two tables were merged, and log graphs were created of species richness based on country and the

pertinent factor. Originally a Poisson model was used, however the data was overdispersed (residual deviance divided by degree of freedom was too high). Therefore a QuasiPossion model was used, with an ANOVA F test.

All data was taken from World Bank (online), with information regarding air travel, GDP, rural population, and urban population (2018). Air transport was recorded as the recoded registered carrier departures (2016) in millions, with the number of HDM species collected from the corresponding country. As HDMs are transferred on clothing, pets and furniture, countries with a higher level of air transport would have higher species richness, as mites would be easily transported from one country to another. GDP was measured as current US$ (2016) in millions, and countries with a higher GDP may be representative of a more affluent country and therefore HDM species richness may be lower and more controlled.

Similarly, rural and urban populations (2016) were recorded, and it is possible that a higher level of urbanization would lead to a lower level of HDM species richness. Countries with high rural populations may not be as prosperous, and levels of cleanliness may be lower which contribute to more mites. In addition, rural areas have higher farming, and although this was not examined in the scope of this database, there are mites transferred from farming equipment into farmhouses. This would increase the number of HDM species collected from that country. Socioeconomic factors and housing characteristics may also affect population density and number of HDMs. Newer houses in good condition identified mites 19% of the time, compared to 40% of the time in poorly constructed single story homes in Turkey (Acican et al., 1993). Mite numbers are also increased by heating using stoves compared to houses with central heating, regardless of whether

the house is made from stone or wood (Stenius & Cunnington, 1972). Mite growth and survival is favoured in carpets (Sharma et al., 2011) and older homes, often due to structural cracks and older furniture (Heikal, 2015, Saleh et al., 2013). More HDMs are also collected from damp homes, due to higher relative humidity (Acican et al., 1993, Heikal, 2015). Statistical results are presented in Table 2.1, and log species richness graphs are provided in Figure 2.5.

Table 2.1. Summary table of species richness based on country and relevant factor which may contribute to HDM fauna (air travel, GDP, rural population, and urban population). Information was taken for a maximum of 13 countries (depending on whether information was provided in World Bank for that location). A QuasiPoisson model with an ANOVA F test was performed.

	Df	**Deviance**	**Resid. Df**	**Resid. Dev**	**F**	**Pr(>F)**
NULL			203	1825.5		
Air travel	1	108.13	202	1717.3	12.504	0.0005090
Country	10	272.38	192	1445	3.1498	0.0009366
NULL			217	1915.1		
GDP	1	40.55	216	1874.5	4.892	0.02809
Country	11	392.23	205	1482.3	4.3021	$8.915e^{-06}$
NULL			217	1915.1		
Rural population	1	75.22	216	1839.8	9.0756	0.002917
Country	11	357.56	205	1482.3	3.9217	$3.589e^{-05}$
NULL			217	1915.1		
Urban population	1	43.04	216	1872	5.1924	0.02372
Country	11	389.74	205	1482.3	4.2748	$9.854e^{-06}$

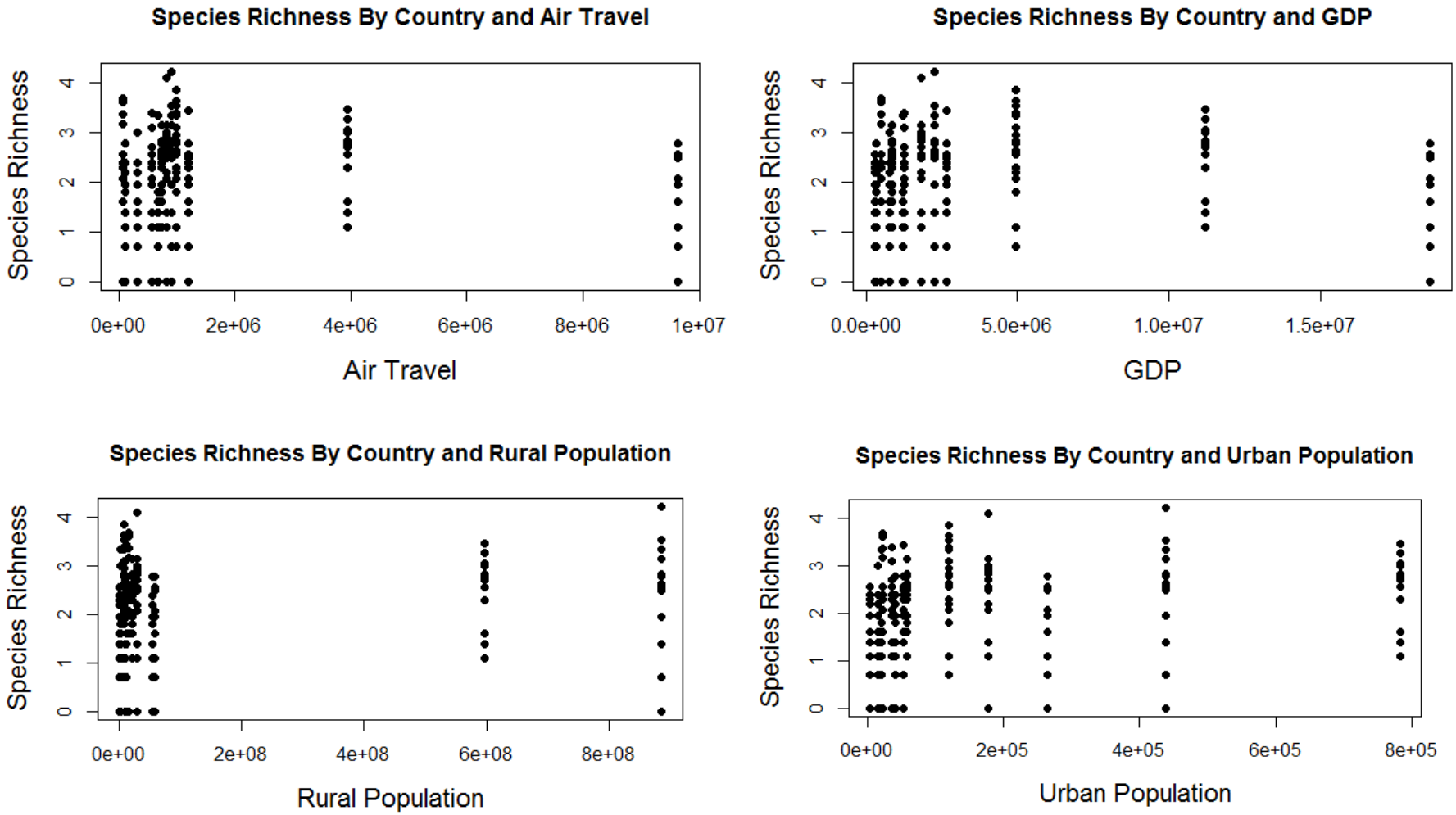


Figure 2.5. HDM species richness in 13 countries with high numbers of publications, and the correlation with air travel, GDP, rural population, and urban population. Each vertical set of dots represents data from a single country regarding the number of species collected within that publication.

Each factor was examined on its own as well as in conjunction with country to determine whether this had an impact on statistical significance. Results from Table 2.1 show that for air travel results were highly significant in both instances ($P<0.001$), for GDP results were significant on its own ($P<0.01$) and highly significant when examined with country ($P<0.001$), for rural populations results were significant on its own ($P<0.01$) and highly significant when examined with country ($P<0.001$), and for urban population results were significant on its own ($P<0.01$) and highly significant when examined with country ($P<0.001$). This indicates HDM species richness is affected both by the number of publications in a country identifying species richness, as well as external factors. As such, when species richness is examined it is necessary to take into account that while HDM fauna varies between countries, this is not the only contributing factor to the species composition per country. It should, however, be noted that data was only provided on World Bank for the year 2016, whereas publications varied from the 1950s to 2017. In addition, the cumulative number of mite species collected from each country was examined; therefore, there is a high degree of overlap of a single species, and it is not representative of the number of different species within a country.

2.4 Conclusions

In total, 347 publications of HDM fauna spanning seven biogeographical regions and 65 countries, identified 531 different HDM species. This encompasses seven countries from the Afrotropical region, three countries from Australasia, 8 countries from the Indo-Malay region, three Nearctic countries, 12 countries from the Neotropics, four countries from the Palearctic East region, and 27 countries from the Palearctic West region. Country was a significant factor with regards to the number of HDM species, however the addition of more countries to the database is necessary to establish whether additional factors, such as level of air travel, GDP, rural and urban populations also contribute to number of species. *D. pteronyssinus*, *D. farinae*¸ *E. maynei*, and *T. putrescentiae* were shown to be the most cosmopolitan and abundant species of HDM. Due to availability, *D. farinae* will be used in future research to examine distribution, as well as population genetic differences (see Chapters 4 and 5).

The aim of this database was to assist future researchers by saving time, money, and effort when analysing HDM fauna. In future, the database could be expanded as more research is accumulated, and studies which were inadvertently overlooked could be added. This database of HDM fauna can be referred to for medical research for HDM mite allergens to identify the most common species collected within the home in a specific country or generalised biogeographical region. Focus could then be directed towards the most abundant species in the attempt to eradicate mites from homes to assist with asthma, allergies and rhinitis. In addition, other factors influencing mite fauna could also be more easily examined with the application of this database. Preliminary research regarding air travel, GDP, and size of rural and urbans populations was conducted, however, there are other factors which may be of interest to other researchers. This includes poverty levels, agriculture, literacy rates, and climatic indicators, to name a few. As mites have such a negative economic impact on agriculture, it may be useful

to explore whether the transfer of HDMs, and the species of mites collected for homes, have a greater impact than originally believed. This could assist with biological control methods to reduce crop exposure to these “pests”. Levels of education could also be correlated with abundance of HDMs. Increased knowledge regarding mite eradication for allergy and asthma suffers could be of use in this instance.

One application of the database is addressed in the next chapter, regarding the concept of a latitudinal diversity gradient, different methods by which this gradient has arisen, and whether there is any correlation between HDMs and a higher level of taxa diversity in tropical climates when compared to temperate regions at higher latitudes. In conclusion, HDMs are found worldwide to varying degrees, and have a medical, economic and forensic importance, as well as having an effect on lives in unknown ways. Therefore, there are many ways in which the data consolidated within this database could be used for future research.

Chapter 3: Biogeographical Distribution of House Dust Mites

3.1 Introduction

Following the creation of the HDM database in Chapter 2, I will further illustrate distribution of HDMs by country and species. This will be expanded upon to discuss the concept of a latitudinal diversity gradient (LDG). This theory states that most taxonomic species have higher levels of diversity in the tropics, compared to temperate zones at a higher latitude. A previous study has been conducted on oribatid mites and their relationship to a LDG (Maraun et al., 2007). Oribatid mites mainly live underground in the soil, where they act as decomposers, by feeding on dead organic material, fungi, and small animals. Examination of 22 islands and 33 mainland geographical areas illustrated that while oribatid mite species richness increased with decreasing latitude, this pattern only continued to warm temperate sites and did not reach tropical locations. There is little known about below-ground taxa and the presence of a LDG. The authors proposed two factors which may contribute to the non-linear pattern they identified in oribatid mites. They believe oribatid mites are generalists with regards to habitat and feeding. They do not have a strong relationship to plant or fungal diversity, therefore there would not be a correlation between plant diversity and mite diversity increasing towards the tropics. In addition, there are minimal differences between the physio-chemical composition of litter in temperate and tropical sites. Food resources are similar for the mites and therefore it is unlikely they would adapt to specific local niches (Maraun et al., 2007).

In this chapter, I will outline some possible explanations for a LDG, and as another application of the HDM database, examine the most highly-published countries identified in the previous chapter. If HDMs were to follow a LDG, there would be greater species diversity in tropical compared to temperate climates.

3.2 Materials and Methods

Two methods were performed to examine whether HDMs follow a latitudinal diversity gradient (LDG). For both methods, latitude was determined on google for a specific region or city (if provided), for a maximum of three locations per study. If mites were collected from more than three locations, the mid-point latitude of the country was used for simplicity. If no region or city was noted in the publication, again the mid-point of the country was used. The average (absolute values, without including the distance north or south) was then taken.

In the first method, a graph of the 13 countries which had a minimum of 10 publications (see Chapter 2) was created in Excel to compare the number of species and the latitudinal distance from the equator. "New species" were plotted for each country, thereby only the number of different species were examined.

In the second method, the 13 countries were examined in RStudio (2015c), and again the number of species and latitudinal distance from equator was determined. This method differed from the first as this method was a cumulative count of number of species across all publications from each country. Therefore, this does not show the number of different species per country, but rather the total number of species which were collected across all publications in each of the 13 countries. Originally a Poisson model was used, however the data was overdispersed (residual deviance divided by degree of freedom was too high). Therefore a QuasiPossion model was used, with an ANOVA F test.

3.3 Results and Discussion

3.3.1 Distribution by biogeographical region:

The number of different HDM species in each biogeographical region was graphed in Figure 3.1. The highest number of species were collected from the Palearctic West region (265), followed by the Neotropical (207) and the Indo-Malay (204). Overall these three regions are highly researched (see database), which would increase the odds of collecting more species and provide more opportunities to identify new mite fauna.

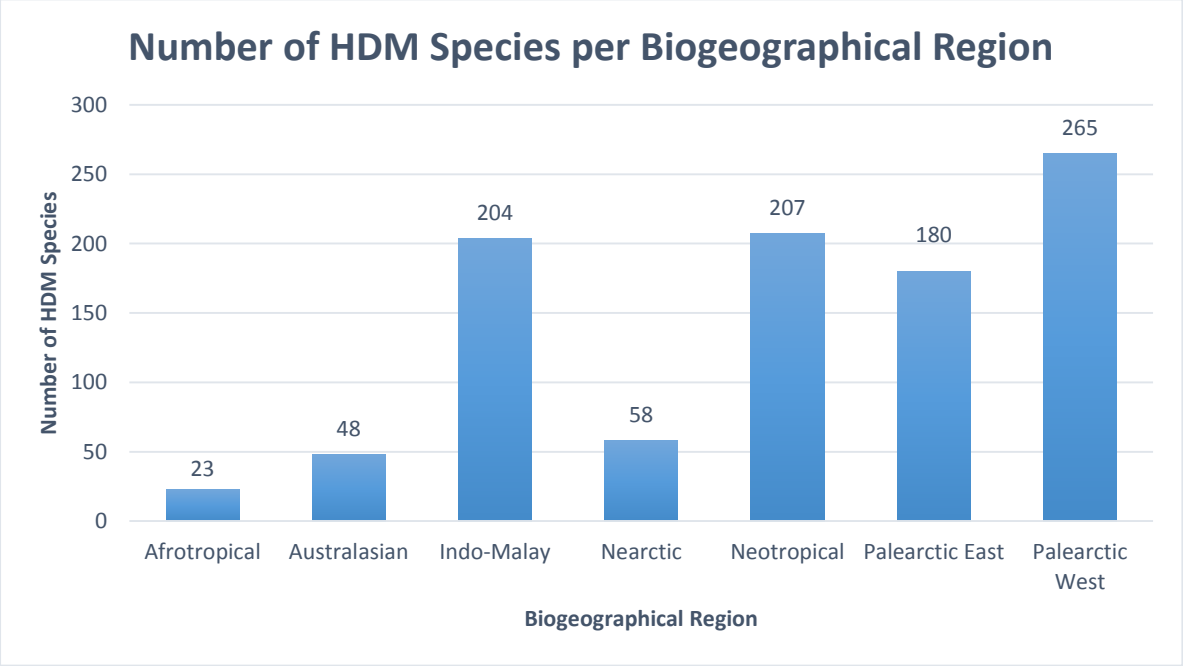


Figure 3.1. Number of HDM species collected from seven biogeographical regions in 363 publications. The highest number of species were collected from the Palearctic West, and the fewest from the Afrotropical region.

3.3.2 Distribution by country:

Two heat maps were generated of HDM fauna using data from the country of mite collection (not the country researchers were based). The first was of the number of publications on HDM fauna per country (Figure 3.2), and the second was of species counts from each country (Figure 3.3). Darker shades either indicate a higher number of publications or a higher number of HDM species within that country. The United States of America had the highest number of

publications (28), followed by Japan (22), Poland (21), and India (20). India had the highest number of species (153), followed by Japan (112) and Brazil (99).

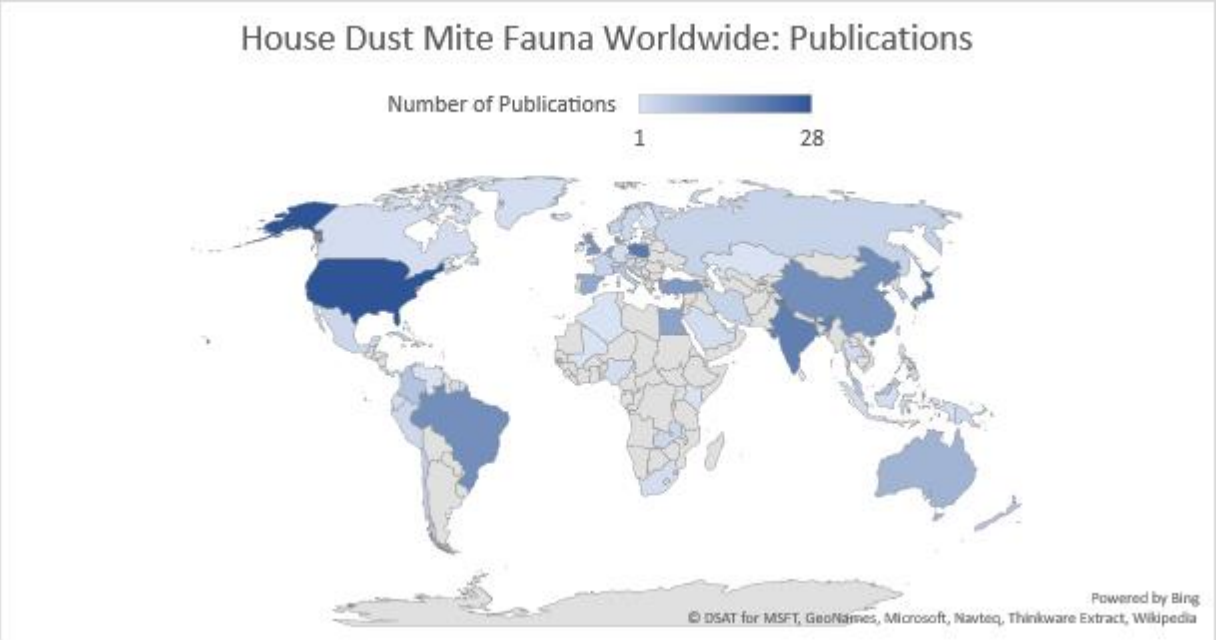


Figure 3.2. Distribution HDM fauna in each studied country based on the number of publications. The highest number of publications were conducted in the USA (28), with a gradient to only a single publication from 16 countries.

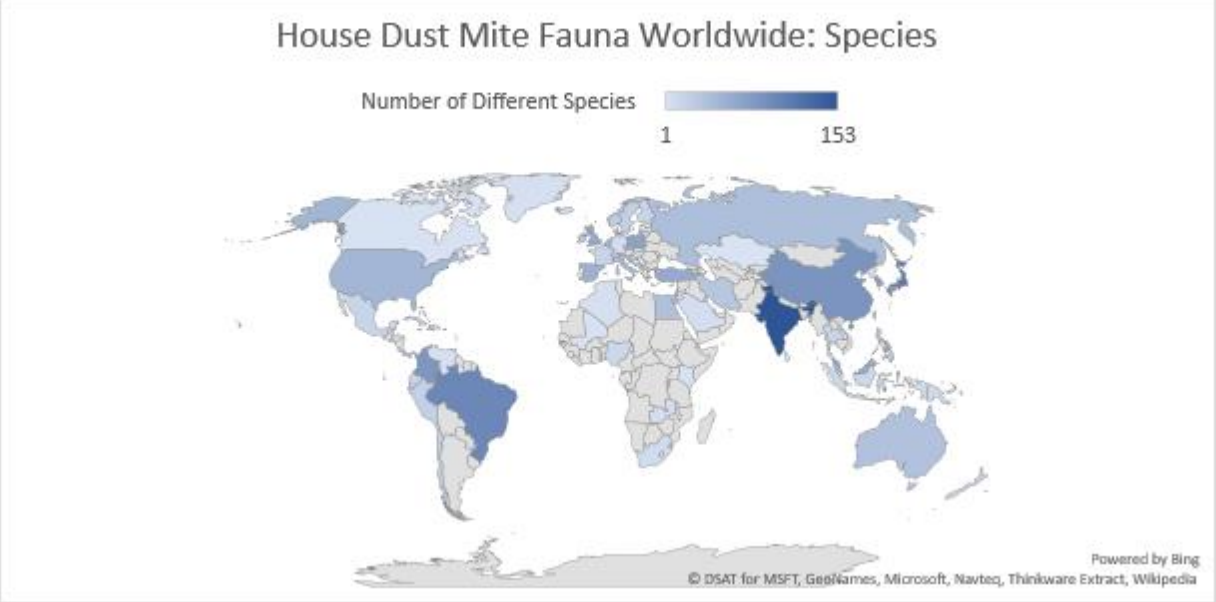


Figure 3.3. Distribution of number of different HDM species collected from each country where there was published research into HDM fauna. The highest number of species were collected from India, Japan, and Brazil.

Next, the 10 countries with the most diverse HDM fauna were graphed in Figure 3.4. Species counts were often highest in countries where the most sampling has been conducted. This suggests that greater sampling effort may be at least partly responsible for the apparent higher species diversity. In addition, many of these countries are in tropical locations, which suggests a LDG in HDMs.

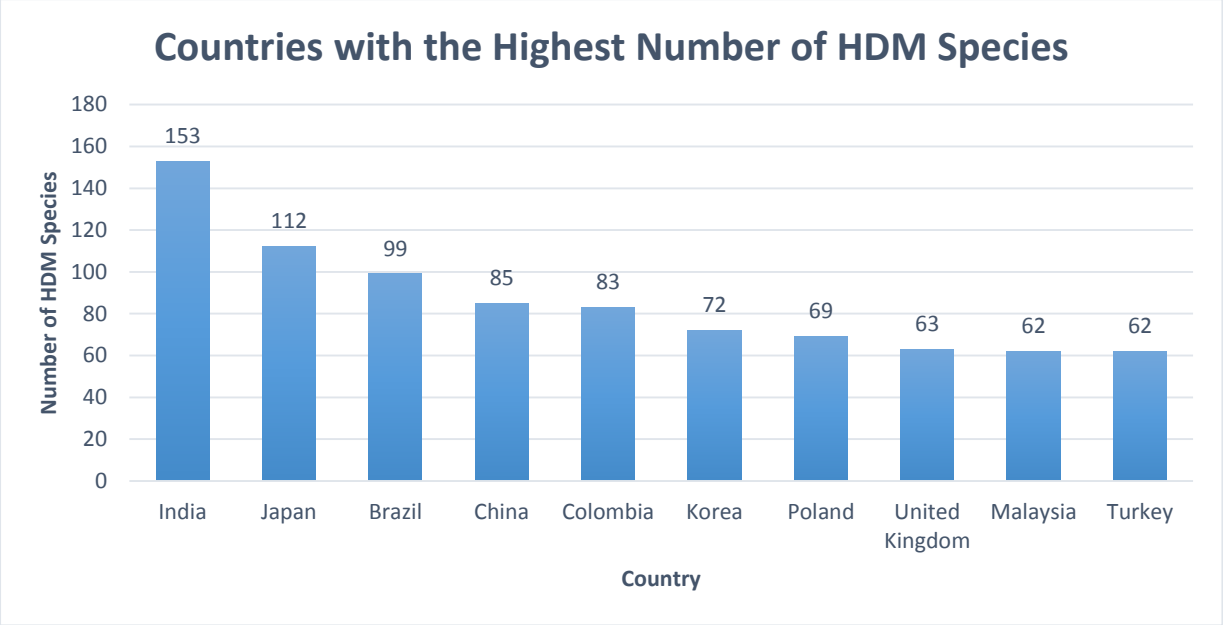


Figure 3.4. The ten countries with the highest number of HDM species, as identified from 363 publications in the HDM fauna database.

3.3.3 Latitudinal diversity gradient:

The new database of mite diversity that I have complied can be used to investigate global patterns of mite biodiversity. As an application for this database, I chose to examine whether HDM fauna follows a LDG. The existence of a LDG can be traced back millions of years, however the mechanism behind this gradient or the reasoning behind why it occurs has not yet been established (Mittelbach et al., 2007). Early explanations for the LDG proposed by Darwin (1859) and Wallace (1878) focused on geographical differences, where temperate regions have a harsh climate for adaption, whereas in tropical regions biotic interactions are enough for adaptation (Mittelbach et al., 2007). There is an overwhelming amount of literature on different

hypotheses for a LDG, and given time constraints, it is not possible to review every theory. Over time, many theories have been proposed, altered, rejected, and combined with other theories. As a result, one single theory has not been accepted as correct. See the following for reviews of some of the theories (Currie, 1991, Schemske & Mittelbach, 2017, Rohde, 1992, Pianka, 1966, Willig & Presley, 2018, Mittelbach et al., 2007, Willig et al., 2003).

One of the main issues surrounding the concept of a LDG besides the number of theories, is that often only one species, or a small related group, has been used to assess the hypothesis (Hillebrand, 2004). This is based on simple correlations between diversity and species richness, and the main focus is often on vertebrate taxa and higher plants. Additionally, there is a bias towards studies being conducted in the Americas (Hillebrand, 2004). Theories centre around one of three possibilities: ecological, evolutionary, and historical (Mittelbach et al., 2007). Ecological hypotheses examine the mechanisms of how species coexist and diversity is maintained, evolutionary hypotheses observe diversification rates, and historical hypotheses focus on how long tropical environments have existed (Mittelbach et al., 2007). Some of the possible explanations for the existence of a LDG will be outlined below.

3.3.4 Pleistocene glaciation:

a. Hypothesis:

Over the last 700 000 years, major ice ages have been interspersed with short warm interglacial periods (such as the one currently experienced) (Hewitt, 1996). Climatic oscillations would not have been consistent worldwide, as features such as distance from the equator, ocean position and currents, continental mass and mountain ranges would have variable consequences (Hewitt, 1996). Pleistocene glaciation is a historical hypothesis centering around past glaciations that created an inhabitable environment for organisms in North America and

Eurasia (Brown, 2014). There was a stronger impact of glaciation on the larger amounts of landmass distributed at higher latitude, in comparison to lower (tropical) latitudes (Lomolino et al., 2009) (as referenced in Wilson & Veraguth, 2010). For example, species in European continental areas were very affected by oceans, deserts, and mountains that acted as barriers to create inhospitable environments during glacial periods (Hewitt, 1996). While these high latitudes were covered with ice, tropical regions were not as affected (Hewitt, 2004).

Pleistocene fossil records illustrate most taxa responded in an independent manner to the changing climate (Graham et al., 1996). Species tended to move south during cooling periods and north during warming periods, where genome mixing may occur (Hewitt, 1996). Species adapted to these large demographic changes, resulting in some populations going extinct, bottlenecks and founder events reducing allelic and overall genetic diversity, and mutations may be lost or spread by selection through populations (Hewitt, 2004). Hybrid zones were formed by the expansion of species from their refugia (Hewitt, 1996). These zones may have protected the integrity of two different genomes until the next glaciation cooling occurred when there would be a decrease in the number of species to its refugia (Hewitt, 1996). In the tropics, conditions were colder and more arid, reducing rain forests and spreading deserts and savannah (Hewitt, 2000). Temperate species could adapt further south than "cold-hardy" species (Hewitt, 2004).

b. Select species supporting this hypothesis:

Table 3.1. Some select species which were outlined in the published literature to provide support to the Pleistocene glaciation hypothesis. Molecular markers dated when phylogenetic events occurred, which illustrates differences in species age between tropical and high-latitude (more glaciated) regions.

Species	Method	Reasoning	Reference
Microtus longicaudus	mt cytB	Similarities in Northern subclades due to recent recolonization	(Conroy & Cook, 2000)
		Deeper among-clade branch lengths near glacial areas due to long periods of isolation	
Syngnathus typhle	mtDNA, nDNA, microsatellites	Northern and Eastern European populations had reduced level of genetic variation; recolonization after LGM	(Wilson & Veraguth, 2010)
		Central populations were highly diverse; phylogenetic structure dates to before LGM	
		Southern Atlantic populations had moderate levels of genetic variation; divergences occurred in the middle of the last glacial cycle	
Chorthippus parallelus	nDNA, mtDNA, rDNA	Illustrated five major geographical regions	(Hewitt, 1996)
		Genetic variation was higher in Southern than Northern populations	
Nearctic and Palearctic freshwater / anadromous fish	mtDNA	Tree topologies deeper for non-glaciated (Southern) species compared to Northern species	(Bernatchez & Wilson, 1998)
		Phylogeny very similar among Southern species whereas glaciated species show decreased consistencies	

c. Issues with this hypothesis:

The advancement of molecular methods has allowed for DNA sequencing to be used to date phylogenetic events (Hewitt, 1996). Regions in the D-loop of mtDNA can be useful in distinguishing between mutations due to these hypervariable regions (Hewitt, 2000). Molecular data using DNA similarities can illustrate from which refugia certain genomes appeared, along with their relation to present-day distribution (Hewitt, 2000). This method, comparative phylogeography, can also be applied to illustrate previous discrepancies between genetic and

phylotypic variation (Bernatchez & Wilson, 1998). Although this is a particularly useful method to illustrate species diversity, there is a large amount of error connected with estimating time based on DNA sequence divergence (Hewitt, 1996). Additionally, there are differences in dating between nuclear and mitochondrial DNA results due to the stochastic natures of the coalescent process (Wilson & Veraguth, 2010). This is particularly problematic as much research relies solely on the mitochondrial results; as such a multi-locus data set is necessary to accurately date species divergence (Wilson & Veraguth, 2010).

3.3.5 Niche conservatism:

a. Hypothesis:

Niche conservatism is an ecological hypothesis, where tropical species are more specialised due to adaptations to biotic and abiotic factors, leading to finely distributed resources (Brown, 2014). It is anticipated that every niche will have particular biotic and abiotic factors crucial to survival, and individuals and populations are not expected to survive outside of their niche (Wiens et al., 2010). Niche conservatism may occur within species, therefore phylogenetic tests are not necessarily the most informative or appropriate. There is no universal test for niche conservatism, and different tests may apply in different contexts (Wiens et al., 2010).

The niche conservatism hypothesis is influenced by the historical climate change which occurred during the Pleistocene glaciation (Andam et al., 2016). As previously outlined, glaciation events prevented speciation at higher latitudes; instead the current species collected from high latitudes are derived from species expanding upwards from lower latitudes (Andam et al., 2016). When early colonizers arrive at higher latitudes, they impose barriers to prevent late-dispersers from entering their habitats (Waters et al., 2013) (in Andam *et al*., 2016). Subsequently, the high latitude populations adapt to their new surroundings over time. They

then put further barriers in place to prevent latitudinal movement across climate zones. This creates specific niches for different species (Waters et al., 2013) (in Andam *et al*., 2016).

b. Select species supporting this hypothesis:

Table 3.2. Some select species which were outlined in the published literature to provide support to the niche conservatism hypothesis. This theory centers on dispersion rates limiting diversity at higher latitudes, as well as contributing factors from the Pleistocene glaciation.

Species	**Method**	**Reasoning**	**Reference**
Streptomyces bacteria	Isolation and characterisation of 924 strains	Limitations in dispersal distance despite the potential for long-range dispersal through desiccation-resistant spores	(Andam et al., 2016)
		Taxon composition varied between the different sites	
Amazonian birds	Phylogenetics of 739 species	Ecological speciation is the driving force behind their diversity	(Salisbury et al., 2012)
Desmognathus and *Plethodon*	mtDNA	Niche conservatism promotes allopatric isolation (isolation of biological populations of the same species) and speciation	(Kozak & Wiens, 2006)
		Vicariance events (evolutionary conservatism in climatic tolerances) resulted in allopatric sister lineages	
		Two of eight pairs of allopatric sister taxa do not support this hypothesis (strong divergence in climatic niche area)	
		Small climatic gradients resulted in highly fragmented populations between mountainous ecosystems and lowlands	

Due to their small size and dispersal tendencies, *Streptomyces* bacteria across North American soils may be most relevant to a potential LDG in HDMs. As *Streptomyces* species form desiccation-resistant spores that can be carried in the wind, it provides an opportunity to test whether limitations in dispersal has a strong effect on terrestrial microbial diversity (Andam et al., 2016). Examination of physiologically similar strains of these bacteria from geographically different yet ecologically similar habitats illustrated a LDG. There were limitations in dispersal and genetic drift resulting in regional diversification of *Streptomyces*, providing support to the

niche conservatism hypothesis. There is a bias towards the tropics with regards to niches categorised by dispersal constraints. Consequently, these areas were colonized following glacial retreat by species expansion from lower latitudes (Andam et al., 2016). These tropical niches decrease dispersal across barriers, resulting in an increase in geographic speciation diversification and a LDG (Salisbury et al., 2012).

c. Issues with this hypothesis:

See Peterson (2011) for a full review of issues surrounding the ecological niche conservatism hypothesis, including problems such as over-interpretation of niche models. Methodologies widely vary, and "niche" can be defined very simply to result in a high level of conservatism (Peterson, 2011).

A similar theory to niche conservatism is the physiological tolerance hypothesis (Currie et al., 2004). This hypothesis states that species exist in the regions they have adapted to, therefore species richness is limited by the number of species which can inhabit these areas. The authors agree that while climatic tolerances do limit species dispersal, there still remain many areas that a species could tolerate the climatic conditions, but have not dispersed to. As such, this hypothesis was rejected on those grounds (Currie et al., 2004).

3.3.6 Tropical conservatism hypothesis:

a. Hypothesis:

To build upon the niche conservatism hypothesis, three ideas were combined: high species richness originated in the tropics and expanded outwards to more temperate zones, extant clades from the tropics existed because there was a greater geographic extent, and many species

and clades are well-designed for tropical locations and would not be able to adapt to colder climates (Wiens & Donoghue, 2004). The tropical conservatism hypothesis assumes a relationship between how long a species has been in an area and how many species are currently present. It states that high species diversity will be in regions with warm temperature and high rainfall, and there will be differences in relative ages and diversities between clades in temperate and tropical locations. Cold climates and niche conservatism prevent tropical species and clades from invading more temperate locations (Wiens & Donoghue, 2004). There should be few transitions between tropical and temperate habitats due to the conservation of environmental tolerances (Kerkhoff et al., 2014).

The tropical conservatism hypothesis is not mutually exclusive; instead it forms a nested hierarchy with the diversification rates hypothesis and the "out of tropics" model (Kerkhoff et al., 2014). In the diversification rates hypothesis, a tropical origin for many lineages is highly likely as these environments are older and more wide-spread than temperate regions (Mittelbach et al., 2007). Palaeontological studies provide support that net diversification rates are higher in tropical climates, and species are more likely to have originated in the tropics and then diversified outwards ("out of tropics" model). Phylogenies can also be used to examine diversification rates through sister clades. Sister clades are of equal ages, therefore any differences between species diversity will be because of diversification rates. Although this method has proved accurate in some instances, sister taxa tend to retain ancestral traits, making latitudinal comparisons difficult (Mittelbach et al., 2007).

b. Select species supporting this hypothesis:

Table 3.3. Some select species which were outlined in the published literature to provide support to the tropical conservatism hypothesis. This theory centers on high species diversity in regions with warm temperature and high rainfall, as well as differences in relative ages and diversities between clades in temperate and tropical locations.

Species	**Method**	**Reasoning**	**Reference**
New World woody angiosperms	Phylogeny of 12 500 species and a fossil-calibrated supertree	Lower phylogenetic diversity in temperate regions	(Kerkhoff et al., 2014)
		Temperate and semi-temperate species were clustered and nested within ancient tropical lineages	
		Evolutionary transitions from tropical to temperate environments were very rare	
North American butterflies	Mapping species richness and phylogenies to subfamily level	Mean root distances (number of nodes separating butterfly's subfamily from base of phylogenetic tree) decrease to the south	(Hawkins & DeVries, 2009)
		More derived clades found in Northern regions, and species in basal subfamilies are in the South	
		Winter cold tolerance is main factor explaining why some groups can permanently inhabit temperate region	
Vertebrate clades	Phylogeny of 12 101 species (ray-finned fish, amphibians, turtles, squamates, mammals and birds in North/South America	Families that evolved in the tropics illustrate strong niche conservatism and many lineages cannot expand into temperate regions (contribute little to temperate biodiversity)	(Smith et al., 2012)
		Terrestrial families with temperate origins can inhabit both Northerly and Southerly habitats (contribute strongly to tropical biodiversity)	
Ants	Online datasets and phylogenies	In temperate zones ant lineages are more phylogenetically clustered	(Economo et al., 2018)
		Diversification rates are similar between temperate and tropical regions	

In addition, clades including modern cycads, palms, figs, onychophorans, caecilians, crocodiles, parrots, trogons, and primates are widely distributed in tropical regions, but have not successfully entered temperate zones (Wiens & Donoghue, 2004). This again provides support that niche conservatism reduces the degree of dispersal. However, this hypothesis has not yet been fully developed, therefore more empirical studies need to be conducted (Wiens & Donoghue, 2004).

c. Issues with this hypothesis:

Some data has refuted a LDG under the tropical conservatism hypothesis. For example, grasses (Poaceae) have old lineages and have been able to adapt to a variety of environmental conditions, including arid and cold climates (Visser et al., 2014). This was supported by distribution data for 11,086 species of grasses which illustrated highest species richness at mid-latitudes and only significantly decreased at northern latitudes above 50^{o}. Continental patterns were also visible, with species diversity significantly higher in South America and Australasia. However, not all grass lineages have high number of species in cooler latitudes, and adaptation to temperate climates occurs slowly, if at all. Therefore these dispersion events from tropical locations happened recently (Visser et al., 2014).

The tropical conservatism hypothesis was also contradicted by the New World snake tribe Lampropeltini (Pyron & Burbrink, 2009). Results showed an inverse LDG, where biodiversity in temperate regions were caused by early colonisers and a longer amount of time for speciation (Pyron & Burbrink, 2009). A futher review of 111 phylogenetic studies of birds, mammals, insects and flowering plants mapped the latitudinal ranges of all taxa to determine which hypotheses contributed to the LDG (Jansson et al., 2013). Diversification rates did not differ between sister lineages in tropical and temperate locations. As the tropical conservatism

hypothesis focuses on fewer clades being collected from higher-latitude regions, this hypothesis was not supported. Instead, the phylogenies illustrated that species originated in the tropics and then travelled outwards into temperate zones ("out-of-tropics" hypothesis) (Jansson et al., 2013).

Additionally, a phylogenetic analysis of Old World frogs (Ranidae) depicted a tropical origin in Africa and Asia before spreading to other geographical areas (Wiens et al., 2009). When diversification rates were examined, either rates were similar between tropical and temperate areas, or the level of diversification was higher in temperate clades than tropical in clades. It is possible that temperate regions have higher diversification simply because the clades are younger than those in tropical zones; however, exclusion of the oldest and youngest clades still provided similar results. This illustrates that historical biogeography needs to be included when assessing LDG hypotheses (Wiens et al., 2009).

A LDG cannot be fully explained simply by the longer existence of tropical environments compared to temperate habitats (Mittelbach et al., 2007). Therefore, it is essential to understand why tropical habitats diversity at a faster rate. The seven mechanisms postulated to explain a LDG due to diversification rates are outlined in Table 3.4. It has been suggested that the tropical conservatism hypothesis would be better explained by the combinational effects of the niche conservation hypothesis (discussed in Section 3.3.3) and the time-for-speciation hypothesis (discussed next in Section 3.3.5) (Wiens et al., 2006).

Table 3.4. Description of seven mechanisms to explain latitudinal variation in diversification rates. For a complete description of mechanisms, see Mittelbach *et al.*, (2007).

Mechanism	**Description**	**Reference**
Genetic drift	Rapid speciation in the tropics is due to low average population density leading to greater genetic drift	(Fedorov, 1966)
Climate change	Resulted from change in Earth's orbit and rotational axis (Milkankovitch cycles) which is greater at high latitudes and favours species with high dispersal potential (habitat range could change to follow favourable conditions)	(Dynesius & Jansson, 2000)
Mechanisms of speciation	Unknown at present whether this differs at varying latitudes	(Mittelbach et al., 2007)
Geographical area	As geographical area increases in size, speciation rates are higher and extinction rates are lower, resulting in differences between tropical and temperate diversification rates	(Terborgh, 1973)
Physiological tolerances and dispersal limitation	There is a reduction in physiological tolerances and limited dispersal abilities in species through unfavourable climatic regions, such as that in lowland tropical habitats due to small temperature ranges (compared to higher latitude temperate zones)	(Janzen, 1967)
Evolutionary speed	Biodiversity is highest at the equator as a result of kinetic effects of environmental temperature on rates of biological processes (higher speciation due to increases in genetic divergence caused by mutation rates and generation times)	(Rohde, 1992)
Biotic interactions	Biotic interactions are stronger in tropical regions compared to temperate zones leading to an increase in species richness	(Fischer, 1960, Dobzhansky, 1950)

3.3.7 Time and area hypothesis (Time-for-speciation effect):

a. Hypothesis:

The time and area hypothesis states that species have been amassed for a longer period of time in tropical regions versus temperate regions ("time-for-speciation-effect") (Mittelbach et al., 2007). This hypothesis focuses on both the Pleistocene glaciations, which affected temperate regions more than tropical equatorial areas, as well as how tropical regions were distributed during the early Cenozoic period. During this period, warmer temperatures meant tropical flora covered a large area outside of tropical latitudes. These mild temperatures were followed by a cooling period, and glacial-interglacial cycles which had a more pronounced effect on species

in higher latitudes (Mittelbach et al., 2007). Ultimately, speciation causes species richness; however little research has examined the amount of time it takes for speciation to occur (Stephens & Wiens, 2003).

b. Select species supporting this hypothesis:

Table 3.5. Some select species which were outlined in the published literature to provide support to the time and area (time-for-speciation) hypothesis. This theory states that the tropical regions have had longer to accumulate species; therefore diversity will be increased in comparison to newer inhabitants at higher latitudes.

Species	Method	Reasoning	Reference
Current tree species	Paleogeographic reconstruction of biomes	Positive correlations between current tree diversity and area-time periods since Eocene, Oligocene, and Miocene	(Fine & Ree, 2006)
		High tropical diversity due to larger biomes compared to temperate biomes which lead to increased speciation / lower extinction rates	
New World leaf-nosed bats	nDNA, mtDNA, RNA	Higher rate of sequence divergence at the edges, versus the central equator with average relative tax age decreasing with increasing distance from the equator	(Stevens, 2006)
		Tropical location likely resulted in more time for species richness to occur	
Hylid frogs	Phylogenetic analysis, ancestral area reconstruction, molecular dating methods, and ecological niche modelling	Illustrated species-rich clades of hylids had a tropical origin with radiation outwards to temperate regions at a more recent date	(Wiens et al., 2006)
		Strong correlation between richness in an area and time when the region was first colonised (regardless of geographic location)	
Emydid turtles	Molecular and morphological divergences; phylogenies	High species richness in Eastern North America compared to other continental habitats	(Stephens & Wiens, 2003)
		Reduced species richness in regions bordering eastern North America likely due to more recent colonisation	
		Despite equivalent local species richness, higher levels of regional species richness in Southeast compared to Northeast (North America)	

c. Issues with this hypothesis:

One of the major issues with this hypothesis is that there is an assumption that rates of speciation and extinction are similar between all regions and lineages (Stephens & Wiens, 2003). This is often untrue, as rates can greatly vary over time, space, and between taxa. While the time-for-speciation hypothesis seems to successfully explain patterns of species richness at large spatial scales, with correlations between regions and the length of time species inhabited this area, its effect is indirect and reduced at a smaller scale. At these smaller spatial scales, the time-for-speciation effect creates species richness patterns through local ecological processes such as extinction, competition, adaption, and predation (Stephens & Wiens, 2003).

Currie *et al*. (2004) analysed three data sets and two speciation rates hypotheses (evolutionary rates and biotic interactions), where speciation is predicted to vary with climate due to differences in evolutionary rates or biotic interactions (Currie et al., 2004). In the "evolutionary rates" hypothesis, higher mean temperatures result in shorter generation times, increased mutation rates, and an increase in physiological processes. This will lead to faster rates of selection and higher speciation rates. In the "biotic interactions" hypothesis, differences in the importance of biotic factors between regions will result in differences in speciation rates. However, there is little data to examine either of these hypotheses accurately and thoroughly at this point in time (Currie et al., 2004).

3.3.8 Species-energy hypothesis:

a. Hypothesis:

The other class of ecological hypotheses involves level of productivity (Brown, 2014). The tropics are an area of high productivity; therefore, this region can sustain more individuals among more species. Since temperature and seasonality is at a maximum in tropical climates,

the level of resources is higher, maintaining stable populations and increasing biodiversity. In comparison, the freezing-cold poles and dry deserts have limited life forms due to inhospitable environments. Net primary production (NPP) is limited by both temperature and water in terrestrial habitats, and by nutrients and solar radiation in marine habitats. Consequently, there are limitations on the total amount of resources available, biomass, as well as the number of individuals which can survive in a single location. A fixed NPP means that either a high biomass with few large organisms, or a low biomass with lots of small organisms can be sustained in an ecosystem. As NPP is temperature dependant, a latitudinal gradient exists for productivity level (Brown, 2014).

In this hypothesis, the increase in species diversity in the tropics is caused by faster evolutionary speed – a result of shorter generation times, faster mutation rates, and increased selection at higher temperatures (Brown, 2014, Rohde, 1992). However, species diversity increases with temperature at a faster rate than the number of individuals and NPP. As a result, productivity is not enough to fully explain the LDG, and instead the direct effects of kinetics are influential on the LDG. In the tropics, increased temperatures lead to higher metabolic rates, ecological dynamics and coevolutionary processes, which all act to main a high level of biodiversity (Brown, 2014).

A model was developed which included temperature in the "energetic-equivalence rule", which states that individual metabolic rates increase with body size (Allen et al., 2002). Body metabolic rates within an individual vary with body size and temperature, therefore the addition of biochemical kinetics of metabolism provides another component to test species diversity. Terrestrial, freshwater, and marine taxa examined across latitudinal gradients in North America and elevation in Costa Rica, all supported an increase in species diversity with environmental

temperature. Biochemical kinetics influence metabolism, and metabolic rates direct an individuals', and populations' resource requirements. Higher temperatures increase the rate of biochemical reactions controlling speciation, thereby increasing species diversity (Allen et al., 2002). To expand upon this theory, Brown (2014) used theoretical and simulation models to create a pattern of beta diversity, where biotic factors prevent species from dispersing into other niches (Brown, 2014). Rates of diversity are therefore increased, whereas rates of dispersal are decreased. A combination of higher rates of evolution and higher rates of Red Queen coevolution have resulted in increased biotic interactions. In turn, "the Red Queen runs faster when she is hot" leads to higher species diversity in tropical climates (Brown, 2014).

b. Select species supporting this hypothesis:

Table 3.6. Some select species which were outlined in the published literature to provide support to the species-energy hypothesis. This theory centers on higher biodiversity due to more species acquiring resources which will maintain stable populations in areas of high productivity.

Species	Method	Reasoning	Reference
Coral reefs	Published diversity at single or multiple sites	Diversity best predicted by mean annual ocean temperature and regional coral biomass	(Fraser & Currie, 1996)
		Suggests regional coral richness is limited by available energy	
Atlantic salmon, brown trout and sea trout	Data sets and ANCOVA to estimate relationship between log(population density) and log(mean body weight)	Negative relationship between log(population density) and log(mean body weight) suggests competition for energy resources	(Bohlin et al., 1994)
		Fish metabolic rate is proportional to (body weight)$^{0.9}$ and total energy flow is constant within a population but varies among populations and within sites in a population	
Extant continental and continental island birds	Distribution maps, and simulations of random dynamics of species ranges	Species richness is highest at the tropics, with peaks in major mountain chains	(Davies et al., 2007)
		High-resolution (1^{o} equivalent) models, most important components for bird species richness is temperature and topographical variability	

c. Issues with this hypothesis:

To expand on the species-energy hypothesis, Evans *et al*. (2005) proposed nine mechanisms to explain positive species-energy relationships at a macro-scale: sampling, increased population size, dynamic equilibrium, niche position, niche breadth, more tropic levels, consumer pressure, range limitation, and diversification rate. Each of the outlined mechanisms overlap with at least one prediction of another mechanism (Evans et al., 2005). The authors note that the most relevant mechanisms are increased population size, niche position, and diversification rate. These mechanisms will not be further discussed in this dissertation, however more detail can be found in their publication. Testing the macro-scale species-energy hypothesis is currently a difficult task. The relationships are broad and general, and no single mechanism has been identified to explain the cause of this hypothesis, in part due to little research testing all concepts. The overlap between predictions has been ignored in some previous studies, detracting from the contribution each mechanism may make on positive species-energy (Evans et al., 2005).

Finally, extant evidence does not support the species-energy hypothesis, where variation in species richness is due to total number of species in the area (Currie et al., 2004). Climate has an influence on net primary productivity (NPP), and the NPP limits individuals per area. Additionally, the changes in organism density due to latitude or productivity are very small, and therefore cannot solely explain the observed changes in species diversity (Currie et al., 2004).

3.3.9 House dust mites and the latitudinal diversity gradient:

a. Most likely hypotheses:

There have been many hypotheses to explain a general LDG across species, and data suggests this arose from a combination of these proposed hypotheses. Research into swallowtail biodiversity found evidence for the diversification rates hypothesis, illustrating two different rates between temperate and tropical species of similar age (Condamine et al., 2012). These diversification rates were highly influenced by glacial climatic rates between tropical and temperate clades. Additionally, plant shifts helped to promote diversification of swallowtails. In extant populations, tropical biodiversity does not necessary come from a tropical clade, and there have been multiple dispersals outwards from the tropics. The conclusions of this study used a multidisciplinary approach, and outlines that ecological and evolutionary theories and their corresponding evidence need to be combined for an enhanced understanding of the LDG (Condamine et al., 2012).

It seems likely that glaciation events leading to climatic oscillations, as well as physical barriers such as oceans, deserts, and mountains had a greater impact on high latitude species. Following the warming periods, species than had to radiate outwards from the tropics to recolonize the temperate zones (Pleistocene glaciation hypothesis). As such, species have been amassed for a longer period of time in tropical regions versus temperate regions (Time and area hypothesis). Species richness is higher in the tropics, extant clades in this area existed due to greater geographic extent, and species are less able to adapt to colder climates (Tropical conservatism hypothesis). The interaction between biotic and abiotic factors made specific habitats adaptable to organisms. This also prevented some organisms which are better adapted to colder climates from moving farther south, and vice versa (Niche conservatism hypothesis). Additionally, there is higher speciation in the tropics due to increases in genetic divergence caused by mutation

rates and generation times. This increase in kinetic energy contributes to high species biodiversity (Species-energy hypothesis). A combination of many factors and overlapping theories, often reliant on the validity of a previous theory, are required to explain the LDG.

b. House dust mites and the latitudinal diversity gradient:

To establish whether HDMs follow the LGD hypothesis, the 13 countries with a minimum of 10 publications (refer back to Chapter 2, Figure 2.4) were first plotted in Excel to compare the number of species and the latitudinal distance from the equator (Figure 3.5).

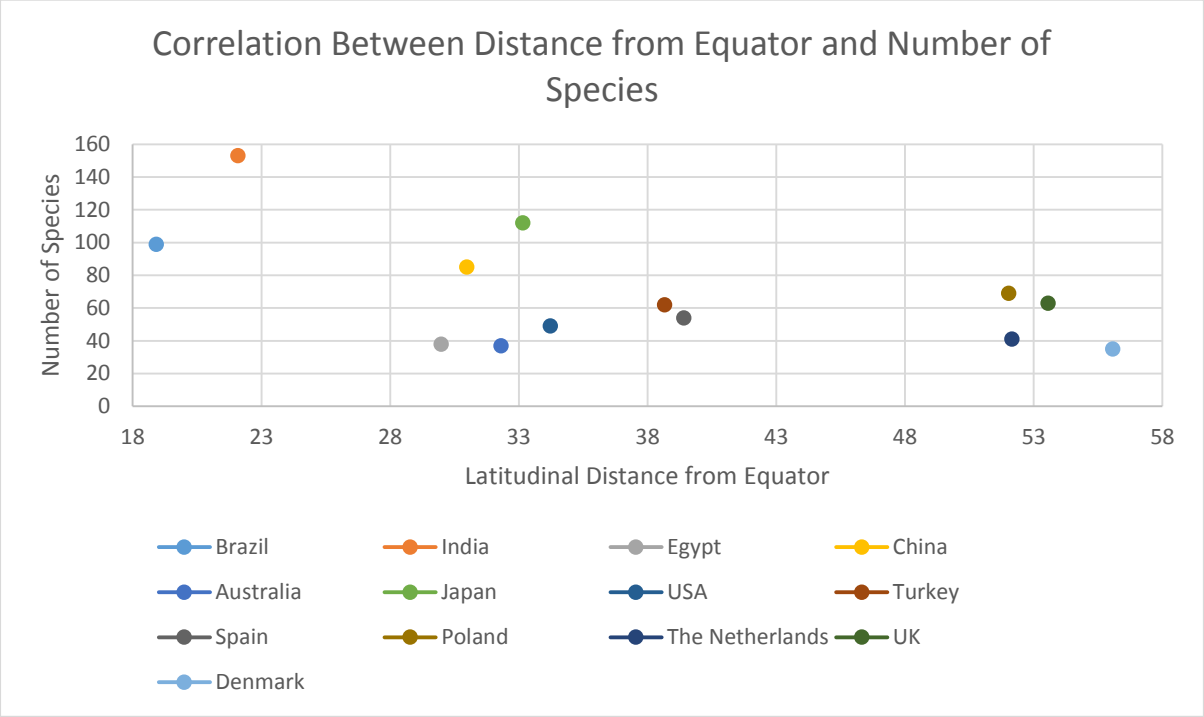


Figure 3.5. Comparison between latitudinal distance and number of HDM species for 13 countries. If mites follow the LDG, there should be a downward linear trend. As distance increases from the equator, number of species should decrease.

Next, the same data was analysed in RStudio (again refer to Chapter 2, Section 2.3.4), however as previously stated, this did not examine the number of different species per country, but instead a total HDM species count. Results are given in Table 3.7 and Figure 3.6, and illustrate that latitude itself is not significant with regards to HDM species richness. However, species richness is highly statistically significant ($P<0.001$) when latitude and country are examined in

conjunction. This illustrates that multiple factors can have an impact on species richness, and there is the possibility that a LDG exists for HDMs. As in the previous chapter, country is a highly significant factor when examining a LDG. This could, however, be due to the locations where the countries with high levels of research being performed. This could inadvertently lead to a country bias suggesting a LDG. To establish whether this is true, more countries would need to be included in this examination, as these 13 countries only suggest that a gradient may exist. If more countries were included, it would become clearer whether a LDG for HDMs exist.

Table 3.7. Summary table of species richness based on country and distance from latitude. Information was taken for 13 countries, and a QuasiPoisson model with an ANOVA F test was performed.

	Df	**Deviance**	**Resid. Df**	**Resid. Dev**	**F**	**Pr(>F)**
NULL			217	1915.1		
Latitude (study)	1	23.99	216	1819.1	2.8755	0.09146
Country	12	421.67	204	1469.4	4.2116	$6.326e^{-06}$

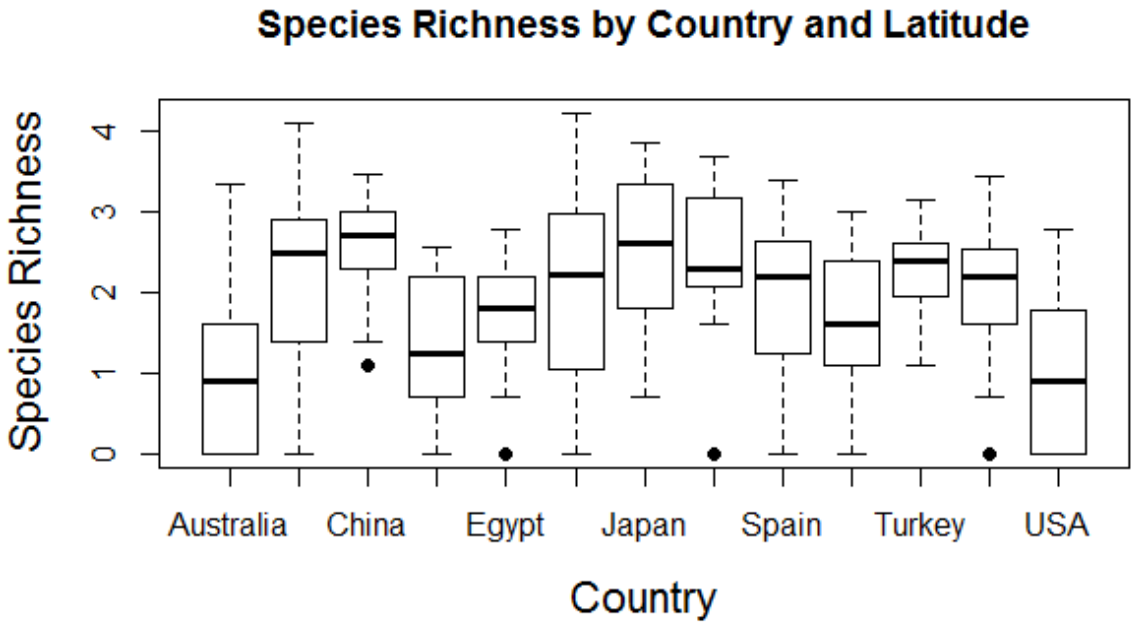


Figure 3.6. HDM species richness in 13 countries, arranged alphabetically, and the correlation with latitudinal distance. The latitudinal distance was determined for each individual study (if given), not the overall country latitude.

One final analysis was performed to examine the existence of a LDG in HDMs. Two countries with contrasting latitudes, Brazil and Poland, were compared. These countries had a high number of publications, and different locations within the country were often noted in the original publication. Therefore exact latitudes per study could be determined, and the corresponding number of species was graphed (Figures 3.7 and 3.8). This illustrated that in tropical locations such as Brazil, there is not necessary a LDG for HDMs; however, in higher latitudes locations such as Poland, there is the potential that HDMs follow this trend. Again, more research in mite fauna at a variety of latitudes and countries would help assess the validity of a possible gradient.

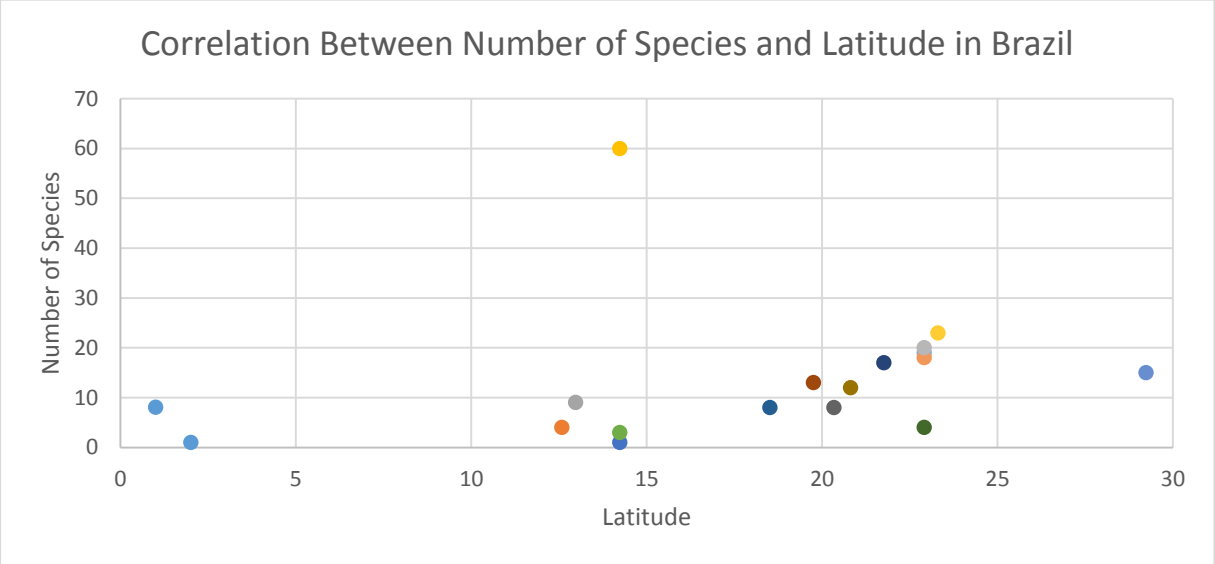


Figure 3.7. Comparison between latitudinal distance and number of HDM species for Brazil. If mites follow the LDG, there should be a downward linear trend, with increasing distance from the equator depicting decreasing number of species. This trend is not seen for Brazil.

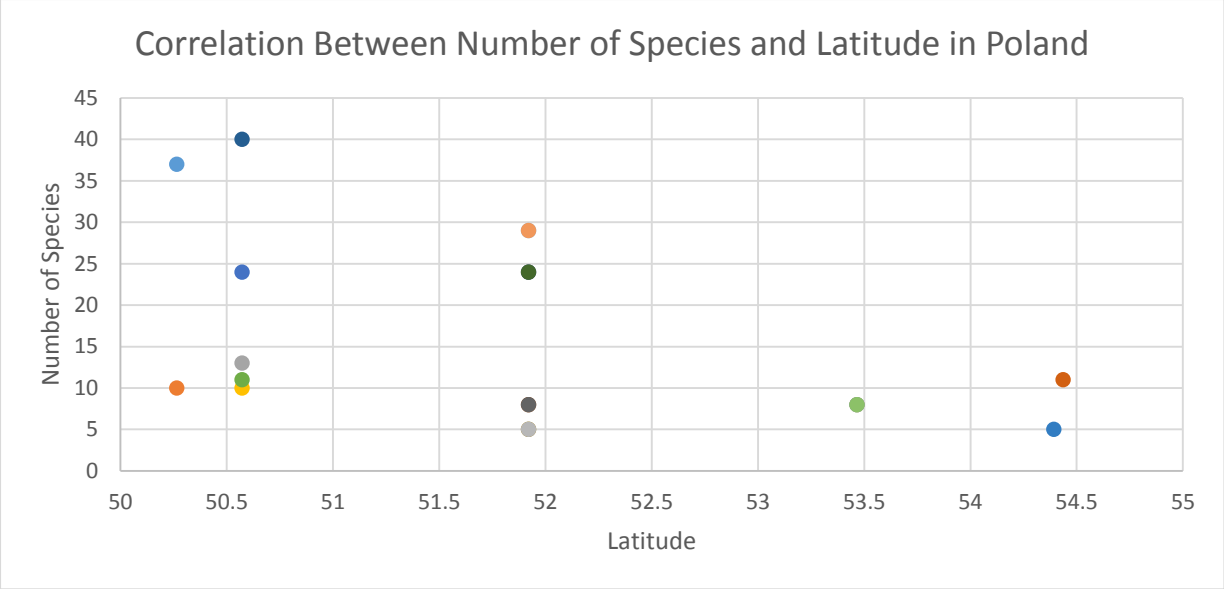


Figure 3.8. Comparison between latitudinal distance and number of HDM species for Poland. If mites follow the LDG, there should be a downward linear trend, with increasing distance from the equator depicting decreasing number of species. This trend is seen for Poland.

It should be noted that my research has examined mites which have been collected from indoor locations. Although these mites may be transferred on humans, pets, or other entities outside, they are not exclusively found outdoors, and for the true HDMs, the outdoors is not their natural habitat. Other research for LDG examines species collected from the wild, therefore HDMs may not follow the same patterns or support the same hypotheses.

With regards to the LDG theories, Pleistocene glaciation likely does not explain mite biodiversity. Mites have very specific temperature and humidity requirements; therefore I find it unlikely that they survived within tropical regions and then recolonized outwards to temperate zones following the last major ice age. I find it most likely that HDMs follow a niche conservatism hypothesis. Each species has specific requirements for their survival. The niche conservatism hypothesis states that every niche will have particular biotic and abiotic factors crucial to survival, and individuals and populations are not expected to survive outside of their niche (Wiens et al., 2010).

As outlined in previous chapters, the natural habitat of HDMs are locations where they feed on fungi, organic material, and bacteria (Klimov & O'Connor, 2013). Over time, mites have phoretically associated with birds and mammals (Colloff, 2009). As such, their relationship with other organisms is likely a contributing factor to their diversity, and it is possible that movement by other taxa would have relocated mites from tropical to higher latitudes. They could have then adjusted their requirements to survive in a specific location, resulting in their survival in house dust.

3.4 Conclusions

There has been a lot of research on HDM fauna published worldwide. All accessible resources were consolidated into a HDM fauna database, encompassing 65 countries from seven biogeographical regions. As an application for the database, the concept of a LDG was examined for HDMs. It is commonly known and accepted that there is a higher number of species in tropical areas, with lowered species diversity in higher latitude (temperate) regions. Although there are many hypotheses, there is no concrete conclusion as to how or why this gradient occurred.

The 13 most thoroughly researched countries with greater than 10 publications were examined using two different methods to determine whether there was a correlation between number of mite species and latitudinal distance from the equator. The results given in Section 3.3.9b suggests that a LDG may exist for HDMs when taking into account both study latitude and the country, with the correlation to species richness. However for this to be conclusively determined, more countries at a wider variety of latitudes would need to be included.

Chapter 4: Method Development and Optimisation of *Dermatophagoides farinae* Extraction Protocols

4.1 Introduction

The American house dust mite *Dermatophagoides farinae* (Hughes 1961) (*D. culinae* De Leon, 1963) was identified as one of the most common species of house dust mite (HDM) worldwide (Chapter 2), therefore it was chosen for further population-level analysis. As with many mite species, knowledge regarding the species population genetic structure is lacking (see Chapter 1). As molecular and population genetics techniques can provide clues to the natural history of under-examined species (Johnson et al., 2004, Hashimoto et al., 2016, Barraclough et al., 2007, Cesari et al., 2009, Faurby et al., 2011, Fontaneto et al., 2007, Gladyshev & Arkhipova, 2010), I aimed to identify genetic differences between populations of the same species using a variety of molecular markers.

One of the main problems with molecular techniques in mites is the low amount of DNA extracted from a single individual (Li et al., 2010a). To provide a context of the low quantities of DNA from mites, the genome size of *D. farinae* has been estimated to be 53.5Mb (Chan et al., 2015), whereas the genome size of a human is approximately 3000Mb (Morton, 1991). It is commonly stated that 50ng of human DNA is required as PCR template; therefore approximately 1ng of high quality mite DNA would be needed as PCR template. As it is often necessary to only have DNA from a single mite sample, combining mites to increase DNA yield is not often an option (Li et al., 2010b). Even if mites are from the same population, they potentially have diverse genotypes. In addition, there could be accidental contamination of another mite species, which would provide inaccurate molecular results for the species of interest (Li et al., 2010b). This chapter will discuss three different extraction protocols which

were optimised for three European populations of *D. farinae* mites, prior to their use as PCR template in Chapter 5.

4.1.1 Mite samples and sequences:

I obtained three European populations of *D. farinae*, and combined information from these with mitochondrial and nuclear sequences available from GenBank (Table 4.1).

Table 4.1. Summary of *D. farinae* mite populations, including country of origin and GenBank accession numbers, where applicable. This includes *D. farinae* samples which were provided for analysis, as well as information obtained from online populations.

ID	Country of Origin	Provider / Identifier	Accession Number	Reference
DF	Poland	Crop Research Institute (Prague, Czech Republic) / Krysztof Solarz	N/A	(Erban & Hubert, 2015)
VI	Unknown European	Victor Iraola (Spain)	N/A	N/A
BH	The Netherlands	Barbara Hart (France) / Marianne van Bronswijck	N/A	N/A
Online CO1 USA	USA	Klimov & O'Connor (Michigan, USA)	GQ465336	(Klimov & O'Connor, 2009)
Online EF1a USA	USA	Klimov & O'Connor (Michigan, USA)	JQ000856	(Klimov & O'Connor, 2009)
Online genome China	China	Chan *et al* (China)	GCA_000767015.1 (KN266307) (KN266725)	(Chan et al., 2015)
Online CO1 China	China	Yang, Cai & Cheng (Shanghai, China)	HQ287786	(Yang et al., 2011)
Online CO1 Thailand	Thailand	Phumee *et al* (Bangkok, Thailand)	HQ823622	(Insung et al., 2015)

4.1.2 Previous extraction techniques:

The first step was to develop and optimise an extraction protocol for this species of mites. To better identify genetic differences between individual mites and to account for accidental

contamination with a different species, single mite extractions were performed when possible. Extraction techniques successfully used for *D. farinae* in the previous publications above are shown in Table 4.2; most of these involve modifications to commercially available genomic DNA extraction kits.

Table 4.2. Comparison of extraction techniques previously used for *D. farinae* mites. There is a minimal amount of information regarding the most successful extraction protocols for HDMs.

Extraction Technique	**Manufacturer**	**Modifications**	**Number of Mites**	**Reference**
Extraction kit, tissue lysis protocol	Qiagen, GmbH, Hilden, Germany	N/A	Not provided	(Chan et al., 2015)
Invisorb SpinTissue Mini Kit	STRATEC Molecular GmbH, Germany	N/A	Not provided	(Insung et al., 2015)
QIAamp DNA Micro	Qiagen	Mite crushed in drop of buffer ATL prior to step 1; pro K added but not mixed; incubation time 24hr; no carrier RNA added; DNA eluted in 30µL buffer AE	Single mites	(Klimov & O'Connor, 2009)

4.2 Materials and Methods

4.2.1 Mite culturing:

Live mites were provided in T-25 cell culture flasks with filter caps. The flasks were placed in plastic containers, filled with damp blue roll and table salt (Figure 4.1). To prevent mites from escaping their enclosure and potentially contaminating other colonies, Vaseline was used to coat the rim of the plastic container and aluminium foil prevented exposure to light. The colonies were reared on refrigerated broken pieces of dry dog food (Pedigree Vital Protection, for adult dogs with chicken), and placed in a closed desk drawer. *D. farinae* mites have previously been identified in dogmeal and yeast in Manchester and on biscuit meal, as well as in house dust (Hughes, 1976). Additionally, they have been cultured in Gainesburger dogfood at room temperature and 75% RH (Hughes, 1976).

Figure 4.1. The mite culturing method used in the Acarology lab at the University of Reading for sustaining *D. farinae* mite colonies. Flasks were kept isolated from other mite colonies to prevent accidental contamination.

4.2.2 Morphological identification:

Dead mites (from The Netherlands and an unknown European origin; see Table 4.1) had already been identified to species level in other acarology laboratories. However, as the live *D. farinae* mite colonies were reared in a lab which also housed other live mite species, there was a possibility of contamination by other colonies. To visually confirm the species of live mites, a morphological identification was conducted by permanently mounting them on glass slides following the method outlined by Krantz *et al.* (2009) (p. 90-92). Individual mites were left in 70% ethanol for one hour before being transferred into tubes pre-filled with 75% lactic acid (Krantz & Walter, 2009). After one hour the tubes were centrifuged and the liquid was carefully removed. The mites were rehydrated with 70% ethanol for one hour, and placed on a glass slide. A drop of Hoyer's mounting medium was added, and a coverslip was carefully placed. Hoyer's medium is an aqueous solution composed of distilled water, gum Arabic, chloral hydrate and glyercin. It is chosen due to ease of use and good optical properties. The coverslip edges were sealed using Glyptal, an insulating paint which provides a flexible and effective barrier against water, necessary when working with the hygroscopic properties of Hoyer's medium (Krantz & Walter, 2009). Slides were viewed under a phase contrast microscope using immersion oil. Hughes' (1976) key for adult Pyroglyphidae was used as a reference to make an accurate identification.

4.2.3 Preparation of mites:

a. Surface cleaning:

When mites are removed from the cell culture flasks, many contaminants such as food, fungi, mould, bacteria, and dead mites are also transferred. Therefore, it is necessary to surface clean the mites to remove these contaminants and to ensure only one mite is being examined prior to DNA extraction. Many modifications (Table 4.3) were made to the original surface cleaning

protocol provided by the Acarology lab at the University of Reading. This was performed in an attempt to elute the highest concentration of DNA possible from a single mite, in combination with modifications made to the DNA extraction protocol. All techniques requiring the visualisation of mites were performed under a stereo-microscope.

Table 4.3. Comparison between the original protocol for mite surface cleaning and the final protocol following optimisation. Additional modifications were made in between the two outlined protocols, in the attempt to increase *D. farinae* DNA yield.

Step	**Original Protocol Surface Cleaning**	**Final Protocol Surface Cleaning**
1	Isolate mites under the microscope using a metal loop to transfer to a wash glass	Place mites in wash glass
2	Transfer mites to 1.5mL tube	Add 1000µL STE+Tween-20
3	Add 1000µL STE+Tween-20 and place into a sonic bath for 280s	Transfer using pipette to new wash glass
4	Pipet wash out avoiding mites	Repeat steps 2 and 3
5	Add 1000µL STE+Tween-20 and place into a sonic bath for 90s	Add 1000µL nfH_2O
6	Pipet wash out avoiding mites	Transfer using pipette to tubes pre-filled with 45µL Buffer ATL
7	Add 1000µL STE+Tween-20 and place into a sonic bath for 30s	Macerate mites using pestle and begin extraction protocol
8	Pipet wash out avoiding mites	
9	Add 1000µL nfH_2O and place into a sonic bath for 60s	
10	Pipet wash out avoiding mites	
11	Repeat steps 9 and 10	
12	Macerate mites against side of tube and begin extraction protocol	

During *D. farinae* surface cleaning, the watch glass of living mites was originally placed in a sonic bath to agitate the solution and remove contaminants. Although contaminants were easily detached from the mites, DNA concentration was negatively affected, particularly as an extended sonic bath killed the mites. Therefore, this process was removed from the final protocol. In addition, the surface cleaning technique involved more washes with both detergent and nuclease-free H_2O, but ultimately experimentation determined that the DNA quantities

following extraction were optimal with fewer washes. When possible, live mite samples were used, as once an organism dies and the decay process begins, DNA will degrade.

b. Mite maceration:

Three methods of mite maceration were attempted: a pipette tip in an Eppendorf tube, a glass rod in a wash glass, and a pestle in an Eppendorf tube. This was conducted to determine which method was most successful at crushing the exoskeleton, thereby allowing the lysing agents to work best. Ultimately the pestle was easiest to use and proved most effective. The number of mites being macerated varied per extraction from one to 42, to establish if more mites resulted in high DNA concentration. Not only did this not have a significant effect on DNA quantity, pooling the mites would also have created an issue with identifying mite populations in future, due to the inability to separate homozygotes and heterozygotes.

4.2.4 DNA extraction:

a. Qiagen kit extraction protocol:

The first DNA extraction method followed that of the Qiagen DNeasy Purification of Total DNA from Animal Tissues (Spin-Column Protocol) (Hilden, Germany, Cat. No. 69504) along with modifications which were suggested by the Acarology lab at the University of Reading as having previously proved successful with mites. Although this technique was successful with living mites, the dead mites (VI and BH populations) had been previously frozen which may have influenced exoskeleton hydration by possibly making it tougher, and lowering DNA quantity. Maceration with a pestle combined with the Qiagen kit and extended incubation time may not have been forceful enough for the dead mites to successfully disintegrate. The

extraction techniques for the Qiagen kit are detailed in Table 4.4, illustrating modifications from the original Qiagen protocol.

Table 4.4. Comparison between the original protocol for DNA extraction using a Qiagen kit and the final protocol following optimisation.

Step	Original Protocol Qiagen Extraction	Final Protocol Qiagen Extraction
1	Place single mite in a 1.5mL microcentrifuge tube. Add 180µL Buffer ATL	Place single mite in a 1.5mL microcentrifuge tube filled with 45µL Buffer ATL
2	Add 20µL proteinase K. Mix thoroughly by vortexing, and incubate at 56ºC overnight until the tissue is completely lysed	Macerate mite using pestle. Add 45µL Buffer ATL and 20µL proteinase K. Mix thoroughly by vortexing, and incubate at 56ºC overnight
3	Vortex for 15s. Add 200µL Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200µL ethanol (96-100%), and mix again thoroughly by vortexing	Add 90µL Buffer ATL and 20µL proteinase K. Mix thoroughly by vortexing, and incubate at 56ºC overnight
4	Pipet the mixture from step 3 (including any precipitate) into the DNeasy Mini spin column placed in a 2mL collection tube (provided). Centrifuge at 8000rpm for 1min. Discard flow-through and collection tube	Repeat step 3
5	Place the DNeasy Mini spin column in a new 2mL collection tube (provided), add 500µL Buffer AW1, and centrifuge for 1min at 8000rpm. Discard flow through and collection tube	Vortex for 15s. Add 300µL Buffer AL to the sample, and mix thoroughly by vortexing. Then add 300µL ethanol (96-100%), and mix again thoroughly by vortexing
6	Place the DNeasy Mini spin column in a new 2mL collection tube (provided), add 500µL Buffer AW2, and centrifuge for 3min at 13000rpm to dry the DNeasy membrane. Discard flow through and collection tube	Pipet the mixture into the DNeasy Mini spin column placed in a 2mL collection tube. Centrifuge at 8000rpm for 1min. Discard flow-through and collection tube
7	Place the DNeasy Mini spin column in a clean 1.5mL microcentrifuge tube, and pipet 200µL Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1min, and then centrifuge for 1min at 8000rpm to elute	Place the DNeasy Mini spin column in a new 2mL collection tube, add 500µL Buffer AW1, and centrifuge for 1min at 8000rpm. Discard flow through and collection tube
8	For maximum DNA yield, repeat elution once as described in step 7	Place the DNeasy Mini spin column in a new 2mL collection tube, add 500µL Buffer AW2, and centrifuge for 3min at 13000rpm to dry the DNeasy membrane. Discard flow through and collection tube. Leave tube open to air dry for 10min
9		Place the DNeasy Mini spin column in a clean 1.5mL microcentrifuge tube, and pipet 40µL Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 5min, and then centrifuge for 1min at 8000rpm to elute
10		Repeat step 9

A variety of factors, some of which had previously been applied to obtain DNA from small mite specimens, were also altered to increase DNA yield during Qiagen extractions. This included the quantity of reagents, the length of incubation, the temperature of the elution buffer, and the incubation period of the elution buffer. The incubation period was extended from one to three hours, to two days, and then later to four days. Often a gelatinous layer formed in the lysate, and to prevent obstructing the spin columns, the tubes were thoroughly vortexed daily. In addition, quantities of buffer ATL and proteinase K were added daily until the gelatine no longer formed. Due to the small size of mites and therefore the low quantity of DNA, only 80µL of Buffer AE was eluted in two steps, instead of the recommended 200µL. A lower volume was eluted to concentrate the DNA within the buffer, and was performed in two steps to prevent the DNA from sticking to the spin column. However, all these changes did not have a significant impact on DNA concentration (refer to Table 4.6).

b. Alternative DNA extraction protocols:

After the Qiagen extraction protocol proved insufficient to successfully extract DNA from dead mites, I modified the technique for DNA extraction from two protocols: the first from stem bark (Novaes et al., 2009), and the other from mature tree leaves (Lefort & Douglas, 1999). Table 4.5 depicts these other two protocols, with both altered for a smaller starting material and did not involve the use of liquid nitrogen to grind the sample. For the stem bark protocol, the "pellet" was only washed once with cold 70% ethanol, and RNase A was not added. Modifications from the original mature tree leaf protocol also include drying the "pellet" at 37^{o}C, and no RNase digestion or column purification.

Table 4.5. Details of the stem bark, and mature tree leaves DNA extraction protocols. These protocols were used to increase DNA yield from dead mites, as the Qiagen protocol was unable to extract any DNA from these *D. farinae* samples.

Step	**Protocol for Stem Bark of Leguminosae Trees Extraction**	**Protocol for Mature Leaves of Hardwood Trees Extraction**
1	Add 100µL of a CTAB extraction buffer (10mL = 0.2g CTAB + 2.8mL NaCl (5M) + 1mL Tris-HCl (1M, pH 7.5) + 0.4mL EDTA (0.5M, pH 8) + 0.2g PVP + nfH_2O to total) and stir on heat until fully dissolved	Add 100µL of CTAB buffer (20mL = 1000µL Tris-HCl (1M, pH 8) + 800µL EDTA (0.5M, pH 8) + 2.8mL NaCl (5M) + 1.6mL LiCl (5M) + 0.2g CTAB + 0.2g PVP + 0.4g SDS) and 1µL of mercaptoethanol
2	Add 2µL of 2% 2-mercaptoethanol, 2µL of Proteinase K (200mg/mL), and 3.5µL of 20% SDS (w/v). Vortex	Vortex 5sec and incubate at 65^oC for 15mins with vortexing
3	Incubate for 60min at 60^oC. Vortex every 15mins.	Add 50µL of cholorform/isoamyl alcohol (24:1) and invert for 5mins
4	Cool tubes to room temperature.	Centrifuge at 13000rpm for 5mins
5	Add 60µL of chloroform/isoamyl alcohol (24:1) and homogenize by gentle inversion for 5mins	Transfer aqueous phase to a new 1.5mL tube and centrifuge for 1min at 13000rpm
6	Centrifuge at 13000rpm for 15mins. Transfer supernatant to new 1.5mL tubes	Transfer supernatant to a new 1.5mL tube. Add equal volume of cold isopropanol and swirl gently
7	Add 14µL of 10% CTAB (w/v) and 28µL NaCl (5M) and homogenize by gentle inversion	Centrifuge at 13000rpm for 5mins. Discard supernatant
8	Repeat steps 5 and 6	Wash with 100µL of 70% ethanol and centrifuge for 1min at 13000rpm
9	Precipitate DNA by adding 1 volume cold isopropanol. Incubate overnight at -20^oC	Remove ethanol and dry pellet at 37^oC on heat block
10	Centrifuge for 10mins at 13000rpm to pellet the DNA. Discard supernatant	Resuspend in 50µL of TE buffer
11	Wash the pellet with 50µL of cold 70% ethanol (v/v), mix, and centrifuge 13000rpm for 5mins. Discard supernatant.	
12	Dry at 37^oC on heat block.	
13	Resuspend in 15µL of TE buffer and vortex	

Table 4.6 outlines modifications to the washing and extraction technique using the Qiagen kit. In addition, spectrophotometer readings are given for DF mites, and one instance of VI mites.

Table 4.6. Yield of DNA protocol development using a Qiagen kit protocol. Modifications to the technique, to increase DNA concentration, are given numerically beside the sample name. Unless otherwise stated, the modification is then used from this point onwards. All buffer quantities are given in µL, and incubation is in days. Numbers in parenthesis indicate how many consecutive days this volume was added. Quantities of Buffers AW1 and AW2 were not included as the volume was consistent at 500µL each. An asterisk (*) besides the sample name denotes negative values. Single mite extractions did not give high DNA yields.

Sample	**# Mites**	**Buffer ATL**	**ProK**	**Incubation**	**Buffer AL**	**Ethanol**	**Buffer AE**	**Yield ng**	**A260**	**A260/280**
DF1	20	45(x2)	5(x2)	1(x2)	100	100	50(x2)	315	0.064	1.905
DF2	20	45(x2)	5(x2)	1(x2)	100	100	100(x2)	390	0.039	1.425
DF3	20	90	10	2	100	100	50(x2)	155	0.031	1.275
DF4	20	90	10	2	100	100	100(x2)	140	0.015	1.065
DF5	20	90	10	1	100	100	50(x2)	230	0.046	2.020
DF6	20	90	10	1	100	100	100(x2)	540	0.054	1.560
DF7/DF8 (1)	20+	45(x2)	5(x2)	1(x2)	100	100	25(x2)	69	0.028	1.838
DF9-DF12 (2)	20+	90(x2)	5(x2)	1(x2)	200	200	25(x2)	105.5	0.042	1.794
DF13/DF15	10	180	20	2	200	200	25(x2)	250	0.101	1.878
DF14	10	90(x2)	10(x2)	1(x2)	200	200	25(x2)	232.5	0.093	1.680
DF16	8	180	20	2	200	200	25(x2)	255	0.102	1.590
DF17 (3)	10	180	10(x2)	1(x2)	200	200	25(x2)	88.5	0.035	1.157
DF18/DF19 (3)	10	360	20(x2)	1(x2)	200	200	25(x2)	136	0.055	1.302
DF20 (3)	20+	360	20(x2)	1(x2)	200	200	25(x2)	216.5	0.086	1.430
DF21 (4)	11	180	20(x2)	1(x2)	200	200	25(x2)	134.5	0.054	3.280
DF22*	12	180	10(x2)	1(x2)	200	200	25(x2)	-3.5	-0.001	0.040
DF23	1	180	20	2	200	200	50+25	78.75	0.022	1.405
DF24	3	180	20	2	200	200	50+25	134.25	0.035	0.910
DF25	5	180	20	2	200	200	50+25	48.75	0.013	0.687
DF26	1	360	40	2	400	400	50+25	75	0.020	0.785
DF27	3	360	40	2	400	400	50+25	132	0.032	1.205
DF28	5	360	40	2	400	400	50+25	183	0.049	1.505
DF29	1	90(x2)	10+10	1(x2)	100	100	50+25	122.25	0.033	1.525
DF30 (5)	1	180	20	2	200	200	25(x2)	78	0.032	0.755
DF31	3	180	20	2	200	200	25(x2)	103	0.041	0.770
DF32	5	180	20	2	200	200	25(x2)	32.5	0.014	0.635
DF33/DF35	1	90(x2)	10(x2)	1(x2)	200	200	25(x2)	120.5	0.048	0.873
DF34	3	90(x2)	10(x2)	1(x2)	200	200	25(x2)	193	0.077	0.910

DF36*	1	90(x2)	10(x2)	1(x2)	200	200	25(x2)	-24.5	-0.010	-0.660
DF37	3	90(x2)	10(x2)	1(x2)	200	200	25(x2)	23	0.009	0.280
DF38	5	90(x2)	10(x2)	1(x2)	200	200	25(x2)	52	0.021	0.280
DF39*	4	180(x2)	20(x2)	1(x2)	200	200	25(x2)	-3	-0.001	-0.040
DF40/DF41	1	90(x2)	10(x2)	1(x2)	200	200	25(x2)	90	0.036	2.080
DF42/DF43	1	180	20	2	200	200	25(x2)	77.5	0.031	2.070
DF44-DF50	2	90(x2)	10(x2)	1(x2)	200	200	25(x2)	126.5	0.051	1.443
DF51-DF56 (6)	2	90(x2)	10(x2)	1(x2)	200	200	25(x2)	73	0.029	1.931
DF57	1	90(x2)	10(x2)	1(x2)	200	200	25(x2)	50	0.021	1.220
DF58-DF64 (7)	2	90(x2)	10(x2)	1(x2)	200	200	25(x2)	53	0.021	1.543
DF65	3	90(x2)	10(x2)	1(x2)	200	200	25(x2)	110	0.044	1.510
DF66-DF73 (8)	2	90(x2)	10(x2)	1(x2)	200	200	25(x2)	159.5	0.064	1.606
DF74*	40	180+90(x4)	20+10(x3)	1(x4)	500	500	25(x4)	90	0.018	-1.395
DF75*	1	90(x5)	10(x4)	1(x4)	500	500	25(x4)	251	0.045	-5.750
DF76*	1	180+90(x4)	20+10(x3)	1(x4)	500	500	25(x4)	-80	-0.016	0.980
DF77*(9)	1	180+90(x4)	20+10(x3)	1(x4)	500	500	25(x4)	88	0.018	-7.165
DF78 (9)	8	140+70+50	15+10(x2)	1+2	285	285	75(x2)	817.5	N/A	0.403
DF79 (10)	8	140+70+50	15+10(x2)	1+2	285	285	75(x2)	837	N/A	0.290
DF80	82	180+90(x2)	20+10(x2)	1(x3)	400	400	25(x4)	464	0.093	2.620
DF81	4	90(x3)	10(x3)	1(x3)	300	300	40(x2)	107.2	0.027	1.015
DF82-DF100	2	90(x3)	10(x3)	1(x3)	300	300	40(x2)	112	0.029	0.000
DF101	2	90(x3)	10(x3)	1(x3)	300	300	40(x2)	234.4	0.059	2.100
DF102-DF104 (11)	1	90(x3)	10(x3)	1(x3)	300	300	40(x2)	189.6	0.047	5.807
DF105	2	90(x3)	10(x3)	1(x3)	300	300	40(x2)	416	0.104	2.060
DF106*	3	90(x3)	10(x3)	1(x3)	300	300	40(x2)	33.6	0.008	-0.460
DF115-DF140	1	90(x3)	10(x3)	1(x3)	300	300	40(x2)	197.6	0.049	1.315
V1141-V1144 (12)	1	90(x3)	10(x3)	1(x3)	300	300	40(x2)	316	0.410	1.833
DF145-DF155 (13)	1	90(x3)	10(x3)	1(x3)	300	300	40(x2)	332	0.444	1.747

(1) Wash 1x (1.5mL tube), 1x (wash glass), 3x (1.5mL tube); buffer AE left 10 mins before elution
(2) Wash 2x (wash glass), 3x (1.5mL tube)
(3) Buffer AE heated this time only
(4) Nuclease free water used for washes
(5) Wash 5x (wash glass); tubes air dry 10 mins after wash
(6) All washes done in same glass
(7) First wash 180s in STE
(8) First wash 90s STE this time only
(9) No sonic bath this time; wash 2x (STE+Tween, 2x (nfH_2O)
(10) First wash 90s STE
(11) Wash 2x (STE+Tween), 1x (nfH_2O); no sonic bath
(12) New pestle each extraction; dead mites this time; wash STE+TritonX
(13) Wash with STE+TritonX this time

DNA was quantified on a DeNovix DS-11FX+, or a NanoDrop Lite spectrophotometer. Both machines work by measuring nucleic acid concentration at 260nm, purity using the 260/280 ratio, and purified protein concentration at 280nm (2015b, 2016). Ideal readings are A260 at 0.1-1.0, and A260/280 at 1.8-2.0. This illustrates the effect of surface cleaning and Qiagen extraction modifications on *D. farinae* concentrations and quality. If the same technique was used for multiple mite samples, an average was taken of the readings, and these values were included in the above table. Maceration was as follows: DF1-DF12 used a pipette tip, DF36-DF39 used a glass rod, and DF13-DF35 / DF40-DF155 used a pestle in a tube. Yield ng/µL was calculated as concentration in ng/µL multiplied by total elution volume (buffer AE). Negative DNA concentrations, or spectrophotometer ratios indicates the presence of contaminants on the machine or in the sample which prevented an accurate reading.

4.3 Results and Discussion

4.3.1 Species identification:

Due to the presence of other living mite colonies in the Acarology lab there was a possibility of contamination. Therefore, a morphological examination was conducted to identify the species of mite that was being analysed from the living colony of mites which originated from Poland. It was particularly necessary to confirm that the species was *D. farinae* and not the closely related sister species, *D. pteonyssinus* (the European HDM). The following image of a mite from this colony, was taken under a Leica stereo-microscope with attached camera (Figure 4.2). Furthermore, the image of the mite used for morphological species identification is provided in Figure 4.3.

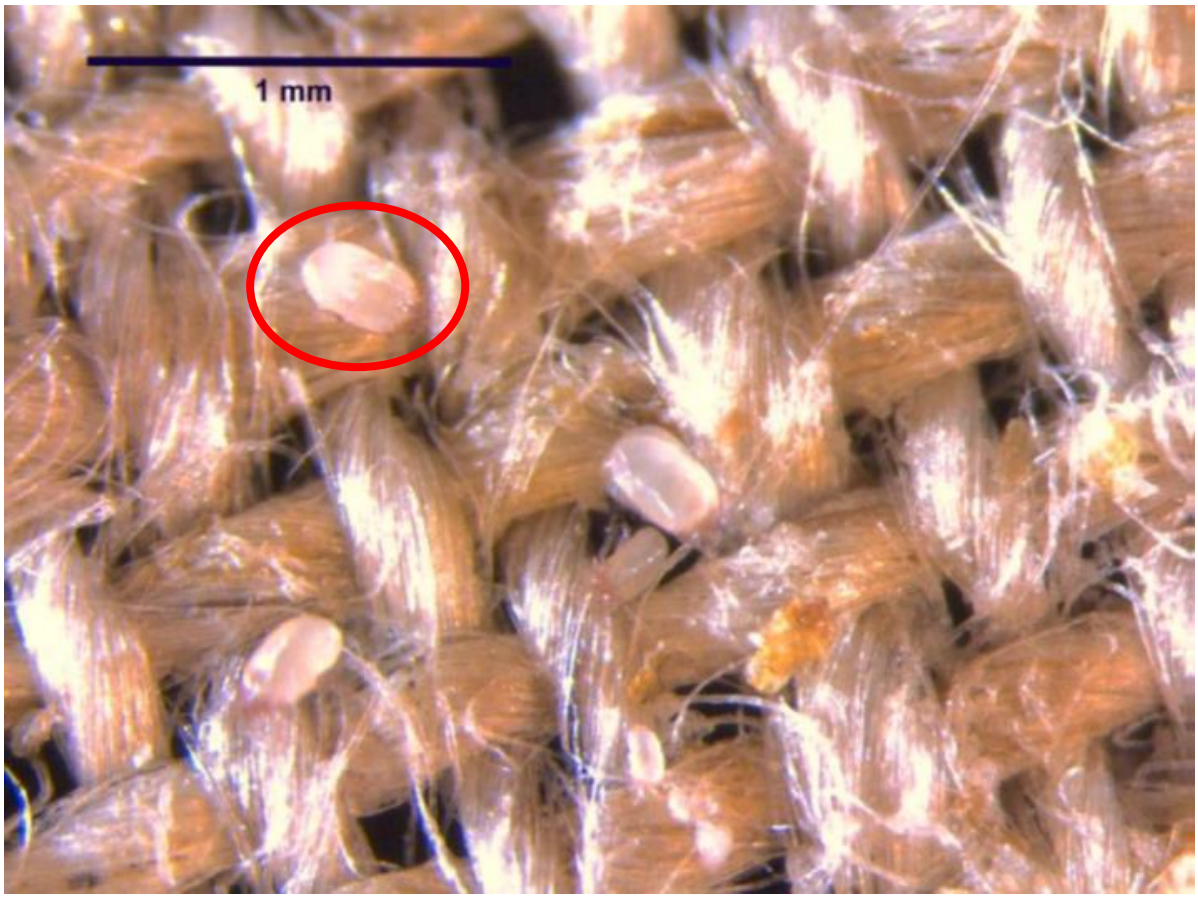


Figure 4.2. *D. farinae* mites on fabric taken under a stereo-microscope (photo courtesy of M. Hani). These mites were obtained from a colony originally from Poland before being cultured in the Acarology lab at the University of Reading.

Figure 4.3. An image taken of a mite from a lab colony at the University of Reading under a phase contrast microscope, identified the species as *D. farinae* using Hughes' (1976) key for adult Pyroglyphidae as a reference.

The mite was identified as a female, due to the lack of a hysterosomal shield, as well as two short setae which replace the vestigial suckers found on tarsus IV of the male (Hughes, 1976). There were two pairs of long setae projecting from the posterior margin of the body, and setae *sc e* were much longer than *sc i*, indicating a Dermatophagoidinae mite (Hughes, 1976). Next, the cuticle between the d_2 and d_3 setae was examined (Hughes, 1976). In *D. pteronyssinus* female mites, the cuticle is longitudinally striated, whereas in *D. farinae* female mites, the cuticle is transversely striated (Hughes, 1976). Upon examination of the mite in question, it was apparent the cuticle which covers the dorsal region of the idiosoma was striated

transversely, indicating a *D. farinae* female (Hughes, 1976). To finally confirm this mite was *D. farinae*, the length of the legs were examined (Hughes, 1976). In *D. pteronyssinus* mites the legs are almost equal in length and width, and this was not true for the mite being identified (Hughes, 1976). In the examined mite, legs I and II were the same length and width, and legs IV were longer than legs III on the tarsus (leg segments) (Hughes, 1976). This is consistent with a *D. farinae* mite (Hughes, 1976).

4.3.2 Mould growth:

During mite rearing, the dog food supplied for the living *D. farinae* mites in the cell culture flasks had an accumulation of mould. Both HDMs and fungi are affected by relative humidity (van Bronswijk, 1981), and the increase in relative humidity in damp dwellings promotes fungal growth (van der Lustgraaf, 1978). If the fungal growth is too high, mites which are susceptible, such as the Pyroglyphidae (including *D. farinae*) will decline over time (van der Lustgraaf, 1978). If contamination were to occur in *D. farinae* colonies, other families such as the Acaridae and Glycyphagidae may outcompete these Pyroglyphidae mites (van Bronswijk, 1981).

An ideal habitat for mite growth was created by the warm temperature in the lab (approximately 25^{o}C), combined with the moisture from the wet blue roll. Not only did this lead to an issue with mite survival, mould may have been extracted alongside the mite DNA, which hindered DNA quantity and risks contamination for PCR. I attempted to rectify the high humidity by reducing the amount of water on the blue roll, which appeared to prevent such rapid mould growth. Following this, the colonies were relocated to a less humid location. However, even after removal of the mouldy food, spores still remained in the cell culture flasks. There appears to be a trade-off between high moisture content increasing mite growth, which is preferable,

and this higher moisture also increasing mould growth, which is detrimental to the survival of the mites. Although the effects this has on further applications is currently unknown, it is possible that mould acted as a contaminant and had a negative impact on quality and quantity of *D. farinae* DNA. This would have had a negative effect on DNA extractions and the subsequent PCR experiments.

4.3.3 Qiagen extraction protocol:

Readings on the spectrophotometer (see Table 4.6), regardless of which machine was used, often provided inconsistent results. This occurred whether the repetitions were taken minutes or days apart. I believed that this was initially due to potential protein residues which had been improperly removed from the machine. However, previous research showed that when the A260/280 ratio was examined over a range of DNA / protein mixtures, there did not seem to be a strong negative effect with the addition of protein until the percentage was approximately 75% (Held, 2001). Therefore, there would need to be a very high concentration of proteins for these results to be seen, which is unlikely.

Due to the nature of the mite sample having a low quantity of DNA, samples needed to be thoroughly mixed through vortexing, and then repeat-pipetted prior to taking a reading. Otherwise the *D. farinae* DNA may not have been taken up by the pipette, and instead the reading was of the elution buffer and any contaminants. This may have contributed to low / negative concentration readings. Factors affecting readings include: the wavelength accuracy between instruments, concentration, types of protein present, and very small differences in wavelength around 280nm (Held, 2001). The machine was always carefully cleaned with nuclease-free H_2O prior to use, in an attempt to prevent contamination with these delicate samples. Although heating samples was not attempted, reports state that heating samples to

37°C for at least 30 minutes, and then thoroughly mixing can ensure that the DNA is completely in solution (2011). In general, optimisation of cleaning and extraction protocols increased DNA quantity. Ineffective maceration may have affected DNA quantity, as could potential variations in individual mite genetic structure. Factors such as genome structure, breeding system, type of reproduction, and differences in ploidy may influence the mite genetic system (Melters et al., 2012). However, there has been minimal research into the number of chromosomes in mites (Oliver & Nelson, 1967), with very little known about the genetic composition of *D. farinae*.

4.3.4 Dead mite extraction protocols:

As previously stated, the Qiagen extraction protocol was only successful with living mites (DF population), however with low DNA concentrations for single mite extraction. Therefore, two other extraction techniques were modified for dead *D. farinae* mites (VI and BH). The first protocol, using a stem bark protocol with minor modifications for mites did work for all three populations, however there were few replicates from each population. This is despite spectrophotometer readings indicating the presence of DNA. In addition, when the technique was attempted a second time, no samples successfully amplified. This was likely caused either by DNA sample decay, due to the mites having been placed in tubes with nuclease-free H_2O weeks prior, or by possible errors during the extraction technique where trace amounts of chloroform/isoamyl alcohol were accidentally transferred. From the stem bark protocol there was a 50% success rate with DF mites, a 17% success rate with BH mites, and a 33% success rate with VI mites (2/4, 1/6, and 2/6 respectively) during PCR amplification (Table 4.7). I wanted a minimum of two replicates per *D. farinae* population to examine genetic differences.

As such, another technique modified from mature tree leaves was conducted. This technique could be completed in a shorter amount of time, and followed similar steps to the stem bark

protocol, however with the addition of lithium chloride. This was the most successful, although it was still not 100% effective. This protocol had a 75% success rate with DF mites, a 100% success rate with BH mites, and a 50% success rate with VI mites (3/4, 4/4, and 2/4 respectively) during PCR amplification (Table 4.7). It is possible that these three extraction techniques were all successful, however varied genetic composition of the mites prevented primer binding. As such, PCR amplification was not 100% effective. An alternative solution was postulated that the mature tree leaf protocol was more successful with *D. farinae* extractions, as the addition of lithium chloride quickly softens cell walls (Hong et al., 1992). This may be necessary when working with the hard exoskeleton of mites.

Table 4.7. DNA quantification on a spectrophotometer following stem bark tree, and mature tree leaves DNA extraction protocols. A single mite was used for each extraction. The sample denoted with an * had a negative DNA concentration, and did not amplify during PCR. It is possible that this sample was highly contaminated and there was no DNA present.

Sample	**Technique**	**A260**	**A260/280**	**Yield (ng)**
DF1	Stem bark	0.630	1.180	294
DF2	Stem bark	0.720	1.190	442.5
BH1*	Stem bark	0.050	-0.100	-147
BH2	Stem bark	0.640	1.260	556.5
BH3	Stem bark	0.870	1.280	1023
VI1	Stem bark	0.400	2.150	178.5
VI2	Stem bark	0.880	1.250	1057.5
VI3	Stem bark	0.790	1.220	1140
DF3	Stem bark	0.570	1.330	1117.5
DF4	Stem bark	0.500	1.570	1525.5
BH4	Stem bark	0.150	3.980	127.5
BH5	Stem bark	0.330	2.780	415.5
BH6	Stem bark	0.270	2.890	522
VI4	Stem bark	0.660	1.390	1419
VI5	Stem bark	0.540	1.300	354
VI6	Stem bark	0.790	1.300	1135.5
DF5	Mature tree leaf	0.350	3.220	530
DF6	Mature tree leaf	0.690	1.420	1335
DF7	Mature tree leaf	0.390	2.580	690
DF8	Mature tree leaf	0.370	3.680	555
BH7	Mature tree leaf	0.560	1.660	520
BH8	Mature tree leaf	0.810	1.360	8875
BH9	Mature tree leaf	0.820	1.330	3295
BH10	Mature tree leaf	0.550	1.960	480
VI7	Mature tree leaf	0.350	2.480	505
VI8	Mature tree leaf	0.810	1.390	7450
VI9	Mature tree leaf	0.500	2.530	2200
VI10	Mature tree leaf	0.380	2.520	435

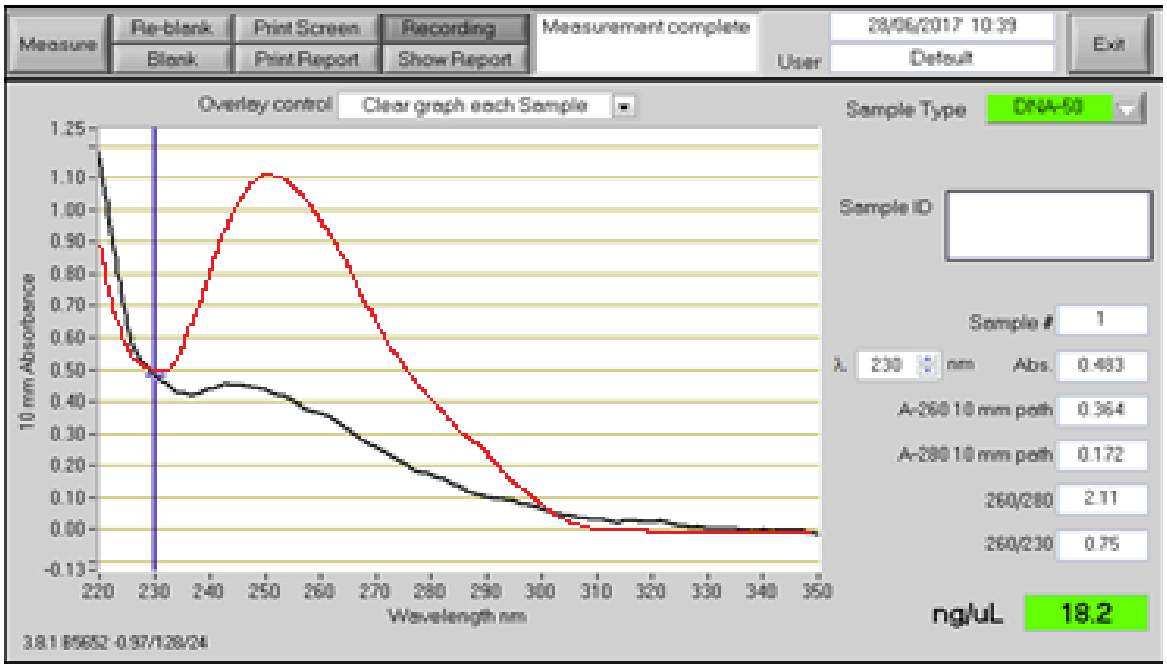
The above results of the mature tree leaf protocol were successful, and these DF, VI and BH specimens could be used as PCR template for further molecular analysis. The results of the PCR reactions of these three populations, along with comparisons to available online sequences (see Table 4.1) are presented in Chapter 5.

4.3.5 Presence of contaminants:

Despite the eventual success of the DNA extraction techniques, the samples seemed to be plagued with contaminants. The A260 and 260/280 readings, which indicate purity of nucleic acid samples, were consistently outside of the normal frame of 0.1-1.0 and a minimum reading of 1.8, respectively. These readings were originally designed to act as a measure of the amount of protein contamination in a DNA sample of interest (2011). However there are additional factors besides excess protein, which could contribute to inaccurate A260 and 260/280 readings. For example, a pH which is too low or too high resulting in destabilisation of the double-stranded DNA structure and thereby influencing absorption, very slight differences between the 260nm and 280nm absorbance values due to very dilute samples which leads to inaccurate ratio calculations, or contamination by proteins, phenols, RNA and other substances which absorb near 280nm and affect the ratio reading (2011). In addition, in mollusc taxa, DNA extraction and PCR amplification is problematic due to the presence of polysaccharides in tissues and mucus (Jaksch et al., 2016). This could inhibit PCR polymerase activity and prevent samples from accurately amplifying (Jaksch et al., 2016). Extraction techniques were compared for RAPD using phenol-chloroform, CTAB, or Chelex 100 illustrating a higher success rate with a double phenol-chloroform extraction (Mikhailova & Johannesson, 1998).

The presence of contaminants was highlighted through the chromatograph images that were provided on the spectrophotometer. Instead of appearing as a discernible peak as anticipated, DNA extracted from *D. farinae* mites resulted in chromatographs that often appeared as either a jagged flat-line across the bottom of the graph near the x-axis, an extremely jagged line at the top of the y-axis that rapidly fell to a straight line near the x-axis, or a graph which did not have the expected peak (Figure 4.4a and b).

A



B

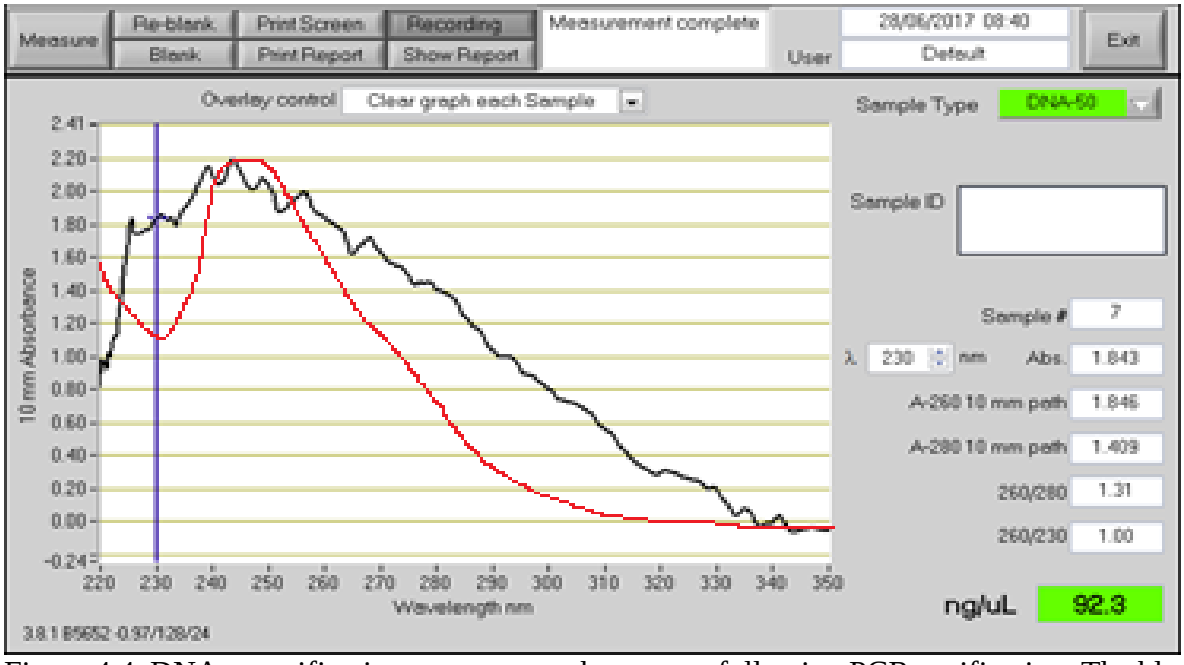


Figure 4.4. DNA quantification on a spectrophotometer following PCR purification. The black lines represent the reading of a *D. farinae* sample, whereas the red lines illustrate an ideal reading.

4.4 Conclusions

Following DNA extraction using three different *D. farinae* extraction protocols (Qiagen kit, stem bark, and mature tree leaves), low concentrations of DNA were obtained. However, it is highly likely that the samples are contaminated. No extraction protocol had a 100% success rate, and a large amount of time was spent attempting new techniques and optimising existing protocols to get the highest quantity DNA possible. The mature tree leaf extraction protocol was the most successful, particularly compared to the Qiagen kit. This may be due to the tree bark and mature tree leaf protocols being developed to break through harder material. The Qiagen kit may have been unable to penetrate the hard mite exoskeleton, despite maceration and an extended incubation period.

Due to the limited knowledge about this species, it was unknown at this point whether genetic differences between populations and individual mites may have contributed to the lack of amplification. Although *D. farinae* mites have proven to be a very difficult species to work with on a molecular level, the best extraction technique for this species was determined. This can be useful for researchers in future to save time and effort on *D. farinae* DNA extraction. However, the low DNA yield suggests that PCR amplification of multi-copy target sequences may be more successful than amplification of single-copy target sequences. This DNA is used a PCR template to provide a basis for analysis of genetic differences between three European populations of *D. farinae* mites (Chapter 5).

Chapter 5: Population Genetic Structure of *Dermatophagoides farinae* Mites

5.1 Introduction

5.1.1 Molecular techniques:

Many methods have been used to examine population structure in different mite species. This includes protein electrophoresis (allozymes), amplification of DNA (PCR), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), microsatellites, direct amplification of length polymorphism (DALP), amplified fragment length polymorphism (AFLP), and DNA sequencing (Navajas & Fenton, 2000). However, some of these techniques are considered outdated, and have more limitations that others (Navajas & Fenton, 2000). A main consideration is that methods need to be adapted to overcome the issues of low level DNA from a single mite. Due to the low DNA yield from single mite extractions, multi-copy target sequences may be more successful for PCR.

5.1.2 PCR of mitochondrial regions:

PCR based techniques require a small amount of DNA template, therefore it can be successful with only a single mite (Thet-Em et al., 2012). To reduce cost and labour, primers can be custom-labelled with a dye allowing multiple primer sets to be combined together in a single reaction (multiplex-PCR) (Culley et al., 2013). The internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA was used to successfully distinguish between populations of *D. pteronyssinus, D. farinae,* and *B. tropicalis* from Thailand (Thet-Em et al., 2012), as well as spider mites (Ben-David et al., 2007). Nested PCR, where generic primers are first used, followed by a second PCR using species specific primers to amplify the region of interest is

useful for small quantities of template DNA to prevent non-target DNA sequences from being amplified and to avoid contamination (Li et al., 2010a, Li et al., 2010b). This has successfully been implemented for ribosomal DNA from single bulb mites (Li et al., 2010a), as well as the 28S ribosomal DNA of single false spider mites (Li et al., 2010b). However, it should be noted that ribosomal DNA is multi-copy, therefore this technique may not be successful with single-copy target sequences. In addition, although there is no annotated genome for bulb mites, the length of a single *Rhizoglyphus robini* mite is approximately 600-940μm (2015a), whereas the length of a *D. farinae* mite is approximately 420 μm (Denmark & Cromroy, 1998). As such, techniques which may be successful on the larger bulb mites may not work with the smaller samples and lower quantities of DNA from *D. farinae* mites.

5.1.3 Amplified fragment length polymorphisms:

Amplified fragment length polymorphisms (AFLP) is a process which involves first digesting genomic DNA with restriction enzymes before ligating adapters to the fragments (Vos et al., 1995). This is followed by two amplifications using primers with minimal selective nucleotides (Vos et al., 1995). This technique does not require large quantities of DNA, can assess an unlimited number of loci, and generic primers can be used eliminating the need for previous sequence knowledge (Weeks et al., 2000). However, AFLP results in largely dominant multiple markers. This makes accurately assessing certain genotypes in diploid or polyploid organisms difficult (Weeks et al., 2000). Heterozygotes cannot be separated from homozygotes, therefore allele frequencies cannot accurately be determined (Lynch & Milligan, 1994). This method has been successfully used to assess genetic diversity and population structure in the phytophagous mite species *T. urticae* and *B. phoenicis* (Weeks et al., 2000).

5.1.4 Microsatellite loci:

Microsatellites are tandem repeats of 1-6bp, evenly distributed though the genome at many diverse loci, and are highly variable with regards to the number of repeats in different individuals (Kantartzi, 2013). In general, mutations in these regions are due to replication slippage or recombination. These markers have a polymorphic tendency, even between closely related lineages, with co-dominance providing discrimination between homozygotes and heterozygotes (Kantartzi, 2013). Despite the many positive attributes of using microsatellite loci, null alleles are common due to high mutation rate in the microsatellite flanking region affecting primer binding (Sun et al., 2012), preferential amplification of short alleles, and slippage during DNA replication (Chapuis & Estoup, 2007).

5.1.5 Nucleotide diversity in mites:

Currently, the Acari is one of the most poorly represented orders with regards to the range of CO1 diversity data published in GenBank (Arabi et al., 2012). A 7.2% within species diversity rate was identified in Tetranychidae mites collected from Europe and North America (Ros & Breeuwer, 2007), and CO1 nucleotide diversity rates ranged from 5% in *T. urticae*, 1.9% in *T. turkestani* (Navajas et al., 1994a), and 0-2.1% in *Mononychellus progresivus* (Navajas et al., 1994b). Furthermore, two morphologically similar species collected from vines in France and Italy were identified as the same species (*Typhlodromus exhilarates*); however there were very low differences in the CO1 region (0.9%) (Tixier et al., 2006). Mites collected from vines less than 200m apart in France had a divergence rate of 3%, which classified these these populations as two different species (*T. exhilarates* and *Typhlodromus phialatus*) (Tixier et al., 2006).

However, the CO1 region cannot always discriminate between populations. Only slight differences were seen between the red and green forms of *T. urticae* following sequencing of

the mitochondrial genome (Chen et al., 2014). The overall finding of low evolutionary divergence was not enough evidence to classify one of the *T. urticae* forms as a new species of mite (Chen et al., 2014). In addition, divergence in the CO1 region in six astigmatid mites (*D. farinae*, *D. pteronyssinus*, *Aleuroglyphus ovatus*, *B. tropicalis*, *E. maynei*, and *T. putrescentiae*) found that distance between species was much higher than within species (Yang et al., 2011). When these sequences were compared to online GenBank sequences, the CO1 region could not be used to discriminate between geographical populations (Yang et al., 2011).

5.1.6 Amplification of *Dermatophagoides farinae* mites:

Primers were designed for the CO1 region of the mitochondrial genome (high copy number; mitochondrial), elongation factor 1 alpha protein (EF1a) (single copy number; coding; nuclear), and non-coding regions of the published genome scaffolds, which were expected to show higher genetic variation (single copy number; non-coding; nuclear) (Kopp & Barmina, 2005). The mitochondrial genome undergoes rapid evolution, therefore illustrating genetic divergence between different populations of the same species (Bowles et al., 1993). As the CO1 gene is essential, universal, and present in high copies per cell, it seemed likely this region would easily amplify in all *D. farinae* samples, from all three populations. This gene is typically highly conserved between members of the same species (Evans et al., 2007, Yang et al., 2011, Kopp & Barmina, 2005), although there may be single nucleotide differences within individuals (Rubinoff et al., 2006). However, the EF1a and non-coding regions of the genome were more likely to show population differences compared to the CO1 region. The transfer of HDMs between locations on humans, pets, furnishings, and in plant soil (van Bronswijk, 1981) could result in panmictic populations, where random mating with other members of the same species leads to increased recombination. However, as the lab mites are isolated and potentially inbred, the same population differences may not be seen as in the wild.

5.2 Materials and Methods

5.2.1 Primer design:

Primers were designed using the online computer program Primer3 Plus (Untergasser et al., 2007). The forwards and reverse primers were examined for self-binding (hairpins) using the online program provided by IDT, as well as binding to each other (primer-dimer) using the online program provided by Thermo Scientific. Primers which bound to themselves at four or more sites were eliminated, as were primers which displayed any primer-dimer to avoid further complications.

Primer sequences were examined using NCBI BLAST (nBLAST and tBLASTx), on nucleotide collection database and whole-genome shotgun contigs for *D. fariane* (2017). This ensured species-specificity, particularly in view of the danger of contamination by human, alternative mite species, or fungal DNA. All primer sequences for CO1, EF1a and non-coding regions, along with primer conditions are detailed below in Table 5.1. Figures 5.1a – d illustrate primer positions in the *D. farinae* genome.

Table 5.1. Primer sequences and conditions for all reactions involving CO1, EF1a and non-coding regions of *D. farinae*. Three primers were designed for the CO1 region, four primers for EF1a, and four primers for non-coding regions of the genome. The sequence, length, melting temperature, GC content, and position in the *D. farinae* genome are provided.

Primer Name	**Primer Sequence**	**Length (bp)**	**Tm (ºC)**	**GC Content (%)**	***D. farinae* BLAST**	**Position**
160F	AGTTGTCTCAGCCTGGTGAT	20	57.3	50	CO1	152-171
1020R	ACCACCCAACATAGTAGCCA	20	57.3	50	CO1	1023-1004
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	26	58.5	34.6	CO1	764-742
05-0812-001 L1	AACCCGTGAACATGCTTTGC	20	57.3	50	EF1a	225-244
05-0812-001 R1	CATTATCGCCAGGGACAGCT	20	59.4	55	EF1a	751-732
05-0812-001 L4	ACCACCGAACCACCATTCTC	20	59.4	55	EF1a	298-317
05-0812-001 R4	CACCAGCCACATAACCACGA	20	59.4	55	EF1a	811-792
Scaffold 551 L1	GTGGACTATGAATGTGCACGA	21	57.9	47.6	Non-coding region	2950-2970
Scaffold 551 R1	GTGTGTGGTGTGTATGTGTTCG	22	60.3	50	Non-coding region	3531-3510
Scaffold 101 L1	GGCGGTGGTAAGAGTGTTGA	20	59.4	55	Non-coding region	12632-12651
Scaffold 101 R1	GCGAGCAAGCAAGCAACAAA	20	57.3	50	Non-coding region	13148-13129

```
   1 taatgcgaaa gagtttaagc ctcttagatt tttattaaaa ttggacgatg attaatatcc
  61 acaaatcata aagatattgc tactttgtat tttgtttttg gggtgtgatc tggtatgttg
 121 gggactaggt ttagaagact aattcgttta gagttgtctc agcctggtga ttttttctatg
 181 gattttgatt actataactc tgttgtaaca gctcatgctt ttattataat ttttttatg
 241 gttatgccta tcatgatagg gggttttggt aatttattgg tgccacttat aattggggct
 301 actgatatgg cctaccctcg tttaaataat atgaggtttt gattattacc cccatctttg
 361 tctcttcttg ttagctctgc ggtggttggt tctggtgttg gtactggatg aactgtttac
 421 ccccccttgt ctaatggtat ctttcactct ggtcctgcag ttgattttgg tattcttagt
 481 ttacatattg caggtgtttc ttctatttta ggtgctatta attttattgt tactattatt
 541 aacataaagg ttgaaggtat agggtgatct agtgttcctt tgtttgtttg atctgttttt
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 661 ttgaccgatc gtaattttaa ttctactttt tttgatcctg tgggaggagg tgaccctatt
 721 ttgtatcaac atttgttttg gttttttggt caccctgagg ttatatcttt gattttgcca
 781 ggatttggta ttattgccca tactgtgagg ttttatagta ataaagttga gccttttggc
 841 tctttaggga tgatttatgc tatagtgtct attgctgtgc ttggatttat tgtttgagct
 901 catcatatat ttacagtggg tttagatatt gatactcgtg cttattttac ttctgctact
 961 ataattattg ctgttcccac tggtgtaaaa gtttttaggt ggttggctac tatgttgggt
1021 ggtaaacttg attttagtcc ttctttttg tggtctatag ggtttgtttt tctctttact
1081 gttggtggtt taactggggt tattttgtct aactcttctt tagatgttag tttacatgac
1141 acgtactatg ttgttgctca ttttcattat gtattgtcga taggtgctgt gtttgctatt
1201 atggcgggaa ttactcattg atttcctgct atttataata ttcctatgaa ccctttatttc
1261 ttaaaaggtc aattttgggt gatattttta ggggttaatt taactttctt tcctcagcat
1321 tttttaggat taaatggtat accacgtcgt tattgtgact atcctgatgg ttttacgtat
1381 tggaatactg taagtagttt agggagattg gttactgtta tatctattgt tttctttgtt
1441 tttattattt gagagggtgt aaggagaggc cgagttgtaa atcagtatac ttctaatcta
1501 actacttcgg agtttttta tgactctcct tccccctccc acaactaatg tgtagacgct
1561 tg
```

Figure 5.1a. Binding positions of CO1 mitochondrial primers, with primer 160F highlighted in yellow, 1020R highlighted in blue, and HCO2198 highlighted in green. Expected amplicon sizes were 160F / 1020R 872bp, and 160F / HCO2198 612bp. GenBank accession GQ465336. Refer to Table 5.1 for additional information.

```
   1 tgggttttgg ataaactaaa agctgaacgt gaacgtggta ttaccattga tatcactttg
  61 tggaaattcg aaacaccaaa atattacgta actgttattg atgctcctgg tcacagagat
 121 ttcatcaaaa acatgattac tggtacttct caagccgatg tggctgtttt gattgtcgct
 181 gctggtactg gtgaatttga agctggtatc tcgaaaaacg gtcaaacccg tgaacatgct
 241 ttgcttgcct atactttggg tgtcaaacaa ttgattgttg gtgtaaacaa aatggacacc
 301 accgaaccac cattctctca agctcgtttc gaagaaatcc aaaaggaagt gtctgcctat
 361 gtaaaaaaga ttggttataa tccggctacc gtcgcttttg tcccaatctc tggctggaac
 421 ggtgacaaca tgttggaccc atcaaccaac atgacctggt ttaaaggatg gtcgattgaa
 481 cgaaaaggac aaaaattcga aggcaaaact ttgttgcaag ctttggatgc ccaagaacca
 541 ccaactcgtc caactgacaa accacttcga ttgccattgc aagatgtgta caagattggt
 601 ggtattggta ccgtgcccgt tggtcgtgtt gaaactggtg ttttgaaacc aggttgtgtc
 661 gttacttttg ctccagctgg tattaccact gaagttaaat ctgtcgaaat gcaccatgaa
 721 gccttgcaag aagctgtccc tggcgataat gttggtttca atgtcaagaa cgtttccgtc
 781 aaggaattgc gtcgtggtta tgtggctggt gatagcaaag ataatcctcc aaagggatgt
 841 gaagaattca ctgcccaggt cattgtattg aatcatccag gccaaatctc caatggatac
 901 actccagtgt tggattgcca cacagctcac attgcttgca aattcaagga aatcaaggaa
 961 aaatgtgacc gtcgttcagg aaaaaaattg gaagatatgc ccaaatcaat caaatcaggt
1021 gatgccgcca ttatcgattt ggcgccaacc aaa
```

Figure 5.1b. Binding positions of nuclear EF1a primers, with primer sets L1 / R4 highlighted in yellow, and L4 / R1 highlighted in blue. Expected amplicon sizes were L1 / R4 586bp, and L4 / R1 453bp. GenBank accession EU152829. Refer to Table 5.1 for additional information.

```
2341 tcatcatcac atcatcataa gaatacaagc acaaatctat tacagactat cggcactggt
2401 gtggtaagtg taaataaaat gtatacatac acatacacat tttacattac aatatatcga
2461 tttacattat ttacatatat tgaatatata atcttcatct attcagattt gttttataca
2521 aattcaaatc tttaaaacaa aaaaaaatat cacatatgga tttgatcgct aagtcaataa
2581 actttttttt tctttttttt gttttgtttt ctattcattt tcttccaatt catagcaaca
2641 actattcaaa tgaatgatgt tcctgaaaca aaactggtcc atatacatag atttatcatg
2701 aagaaaaaaa attcatgata ttcaatcttc acttgaacat acatcaatct gagataacga
2761 aaaaaaaaga aaaaagaaaa atcattcttt tttttcgtca tcgtaacaaa aatctacatt
2821 tatgtccacg ttcaacacaa acgcacacac acacacattt tatgcaaaat tctccaaaaa
2881 aagaaagaaa aaaagaaatt tgaccaaaga attgacaaaa aagtattatt attatttgaa
2941 ccatttaaag tggactatga atgtgcacga tcaaacatct ttatcagcaa atgaaaaaaa
3001 aagaaaaaga aatatggcat gatatttgga catttttttcg gtacaatgat atgaacgata
3061 aataaaatga aactcaatga tgaattttca aacgtgatat tcaacatgat catcatttat
3121 atatgaagcc atatcatgtc ataatgttca gttttcattc attgaaaaac atgaaaccaa
3181 aacataatta cttaatcatc atatatatat tgattataaa tttctatttc gaaagccgag
3241 ccaatcaatc aatatatttt cgattgatac attttaataa ctactaacct tcatacacac
3301 acataaatac acttttatgc caaaaaaaaa acattaaacc actcaatcaa catgaaattt
3361 tcttttcaca tttcggctca ctaaaacgat atagaacgat tatatttgta ccgagattga
3421 tgagatagtg ttcaccaaaa aaaaaattgc tcattcaaat taatattatt tccaataatt
3481 taatccaaat tatcaattta tcaatcaatc gaacacatac acaccacaca cacacacaat
3541 cacaagttat tcaatggatg attttttttc tttcttgttc tttttttttc ttctctcaca
3601 aattcatttg caacaacaaa aatcactaaa aaggttatcc acatttaaca tttcattcat
3661 cacattttac gtaatttaag tgttttcaaa atgaatattt ttttaattgt tatcgctatt
3721 tatttgttaa tccgtttatt gatgatcata attatttgat tgatttattt cgatttttct
```

Figure 5.1c. Binding positions for non-coding scaffold primers 551, with primer sets L1 / R1 highlighted in yellow. Expected amplicon sizes were L1 / R1 582bp. GenBank accession KN266726. Refer to Table 5.1 for additional information.

```
12181 tgttccagaa cgatttttat ttatttgttt gtgtttgcta tgcgaacaac aacaacaaca
12241 acaacaacaa caaaacaaca caacatcatc agtatcgggc cgtcgtcgta caataagtta
12301 atttagaatt attaccatcc catccaatat tcttgttcta ttcgacaaca ttagacatca
12361 acgataaaag aacaaattaa tctaatctaa tagtaatggc aatactcttg ttgtcttctt
12421 gtctattttg acacattttt ttccaaacgg ttatctattt taatttaatt tctctgtata
12481 tgagtataag ggtagatgga aaacgaaaca acaacaaagt cgatgatcat ctttttgagc
12541 gtgaattaaa ttttttaagt ttttcatcat tcaaaaacat taatttgaaa aaaattgtgg
12601 tttccttaca cactttggtc tctagtgaac aggcggtggt aagagtgttg aaatttcacc
12661 gtgatcgatg gttttaaagt tttgaatcag ctacattata aaacacaaga tgacagccat
12721 accaatatgg aatatggtac caccagacat cgtgtgtata taaaaataaa aaacataaat
12781 tgaaatgaca gacaaaaaaa gaactattca ttcttggtca atctatgtat cataccagcg
12841 acaaacaaat cacacacaca cacaaacaca aggcaatggg caaacatttc gtttggatga
12901 atctttgaat ctacctacct tctggaatat atatagacat acctgatgca ttatttatta
12961 tcatacaccc gaattttggt cgaccaatag ttatatgttg ttgttggtga ataaagtaat
13021 ttcattgctg cctgccagct tgcctacctc aaacatggtc gatccattct gttttttttt
13081 tgttttttttt ttttggtatt tagatcccgc tggatttgct gtcgacttt tgttgcttgc
13141 ttgctcgcag gtttctcttt catctttttc tttcagaatg aattccggtt ctcattcatt
13201 catttctatt cttgacaatt tgtatttgaa tgccatcact tttcgaatga tgatgatgat
13261 gatgatgaac accaatggtt gtgtttatca atttgacatc tacacaaata catcagcaat
13321 acctacacaa accgaattcg aatgataatg acgatgacga tgacgatgat gatgaaaaaa
13381 gaggcagcct accgaatgaa atgttttaaa ttattaataa tcatatatat caagatggaa
```

Figure 5.1d. Binding positions for non-coding scaffold primers 101, with primer sets L1 / R1 highlighted in yellow. Expected amplicon sizes were L1 / R1 517bp. GenBank accession KN266307. Refer to Table 5.1 for additional information.

5.2.2 General PCR parameters:

Unless otherwise stated, the following PCR parameters, thermal cycler settings, and gel electrophoresis conditions were used in all experiments. DNA for DF, VI and BH populations were extracted using a stem bark or mature tree leaves protocol, with the positive control (DF population) extracted using a Qiagen protocol (refer to Table 4.7). Each 25µL reaction contained: 12.5µL Go Taq Green Master Mix (Promega); 8.5µL nuclease-free H_2O; 3µL DNA; 0.5µL forwards primer (10µM); and 0.5µL reverse primer (10µM). Thermal cycler conditions were as follows: heat lid to 110^oC; 95^oC for 10mins; 30 cycles of 95^oC for 45sec, 54^oC for 45sec, 72^oC for 90sec; hold at 72^oC for 10mins; hold at 4^oC for infinity. Samples were run on a 1.2% gel using gel red dye for visualisation for 45mins at 90 volts and 400 amps before visualising on a Syngene G: Box gel doc system.

5.2.3 Mitochondrial CO1:

Semi-nested PCRs were performed, with the first reaction using primers 160F and 1020R, and the second reaction using primers 160F and the universal mitochondrial primer HCO2198, which illustrated a few single nucleotide polymorphisms to the online *D. farinae* sequence. The PCR product from the first semi-nested PCR was diluted 1:800 with nuclease-free H_2O, and 3µL of the diluted product was used as the DNA template for the second semi-nested PCR. Thermal cycler conditions for the second PCR were as detailed above, with modifications to the annealing temperature as follows: 30 cycles of 95^oC for 45sec, 52^oC for 45sec, 72^oC for 90sec.

5.2.4 Nuclear EF1a:

Only DNA samples which had previously amplified during the CO1 reactions were used for EF1a nested PCRs. Samples which did not amplify for mitochondrial sequences were not likely

to have a high enough concentration of nuclear DNA, and were therefore omitted. The first part of the nested PCR used primers L1 and R4, and the second part of the nested PCR used primers L4 and R1. The PCR product from the first nested PCR was diluted 1:800 with nuclease-free H_2O, and 3µL of the diluted product was used as the DNA template for the second nested PCR.

5.2.5 Two non-coding regions:

Primer set one (551 L1 / 551 R1) was designed to amplify a non-coding sequence of approximately 550bp in the published scaffold number 551 given online. Primer set two (101 L1 / 101 R1) was designed to amplify a non-coding sequence of approximately 500bp in the published scaffold number 101 given online. These regions were selected after determining in NCBI that they were not coding regions in other species which may accidentally amplify (specifically mites, bacteria, and human). Thermal cycler conditions differed from previous reactions, with parameters as follows: heat lid to 110°C; 95°C for 10mins; 40 cycles of 95°C for 45sec, 54°C for 45sec, 72°C for 90sec; hold at 72°C for 10mins; hold at 4°C for infinity.

5.2.6 DNA purification and sequencing:

PCR purification was performed using QIAquick PCR purification kit protocol by Qiagen, following manufacturer's instructions (Hilden, Germany, Cat. No. 28104). After purification, DNA samples were quantified on a spectrophotometer, and sent for sequencing using Eurofins Genomics Mix2Seq, as per manufacturer's instructions (Ebersberg, Germany). Templates consisted of 15µL purified DNA, 2µL of primer (10µM) with nuclease-free H_2O to a total volume of 17µL.

Sequences were analysed using Geneious v6.1.8 (http://www.geneious.com) (Kearse et al., 2012), and sequences with unclear nucleotides were eliminated. Ends were trimmed based on

the clarity of the nucleotide bases, the reverse complement was taken of the right primer sequence, and a consensus was taken between the forwards and reverse primers for each sample. Only samples which had a clear reading of both the forwards and reverse primers were used in the final analysis along with any corresponding online sequences.

The aligned sequences were exported into DnaSP v5.10.01 for further analysis of nucleotide diversity (Librado & Rozas, 2010). Polymorphisms were highlighted and compared with the chromatographs (Geneious) to make a judgement call on the nucleotide in question, and sequences were amended if necessary. Number of polymorphisms (S), nucleotide diversity (π), θ (per site) from Eta, and Tajima's D were calculated for the CO1 region only, as there were not enough sequences for analysis of the other regions.

A phylogeny of the *D. farinae* mitochondrial CO1 region was created of 20 sequences, combining the aforementationed aligned sequences from three lab populations, online CO1 regions from Thailand, USA, and Canada, as well as the two other main species of HDMs (*D. pteronyssinus* and *E. maynei*), and two outgroups (*Acarus siro* and *Tyrophagus putrescentiae*). The tree was inferred using an MCMC method implemented in BayesPhylogenies (Pagel & Meade, 2004). The model GTR model with gamma rate heterogeneity was selected using a stepping stone sample (Yang, 1994). Three independent analyses were run to check for convergence, and the chains were run for 11 000 000 iterations, with first 100 000 used for burn in. All chains converged to the sample place. The chain was sampled every 10 000 iterations to reduce auto correlation to 0.025. The final sample constisited of 1000 trees.

5.3 Results and Discussion

5.3.1 Mitochondrial CO1:

Semi-nested PCRs were performed for the mitochondrial CO1 region using primers 160F/1020R and 160F/HCO2198 following a stem bark extraction protocol (refer to Table 4.7).

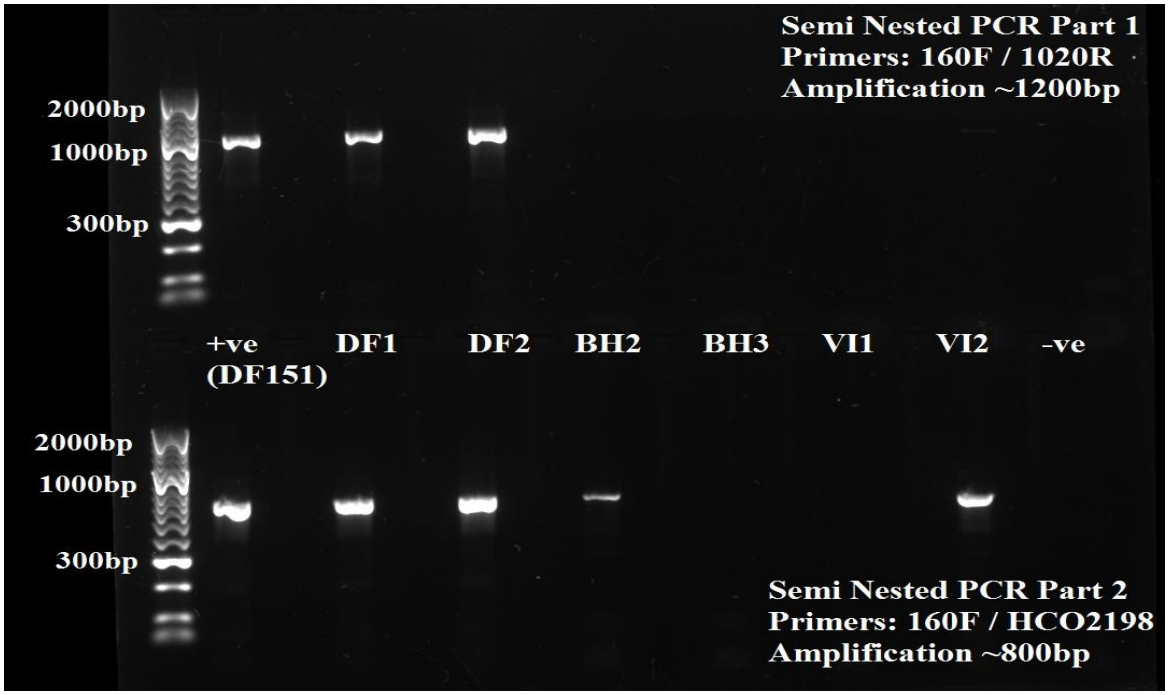


Figure 5.2. Semi-nested PCR of mitochondrial CO1 in *D. farinae* illustrated an observed fragment size of approximately 1200bp and 800bp, respectively, with amplification of samples DF1, DF2, BH2, VI2, and the positive control DF151.

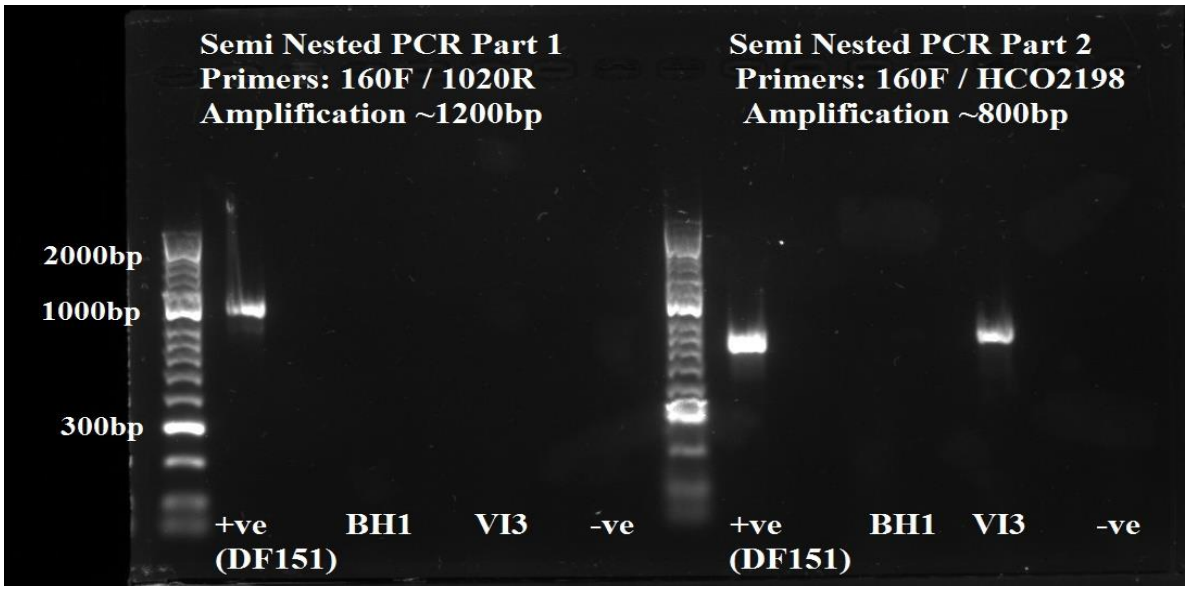


Figure 5.3. Semi-nested PCR of mitochondrial CO1 in *D. farinae* illustrated an observed fragment size of approximately 1200bp and 800bp, respectively, with amplification of samples VI3, and the positive control DF151.

To obtain enough replicates per *D. farinae* population to examine genetic differences, another semi-nested PCR was performed for the mitochondrial CO1 region following a mature tree leaf extraction protocol (refer to Table 4.7).

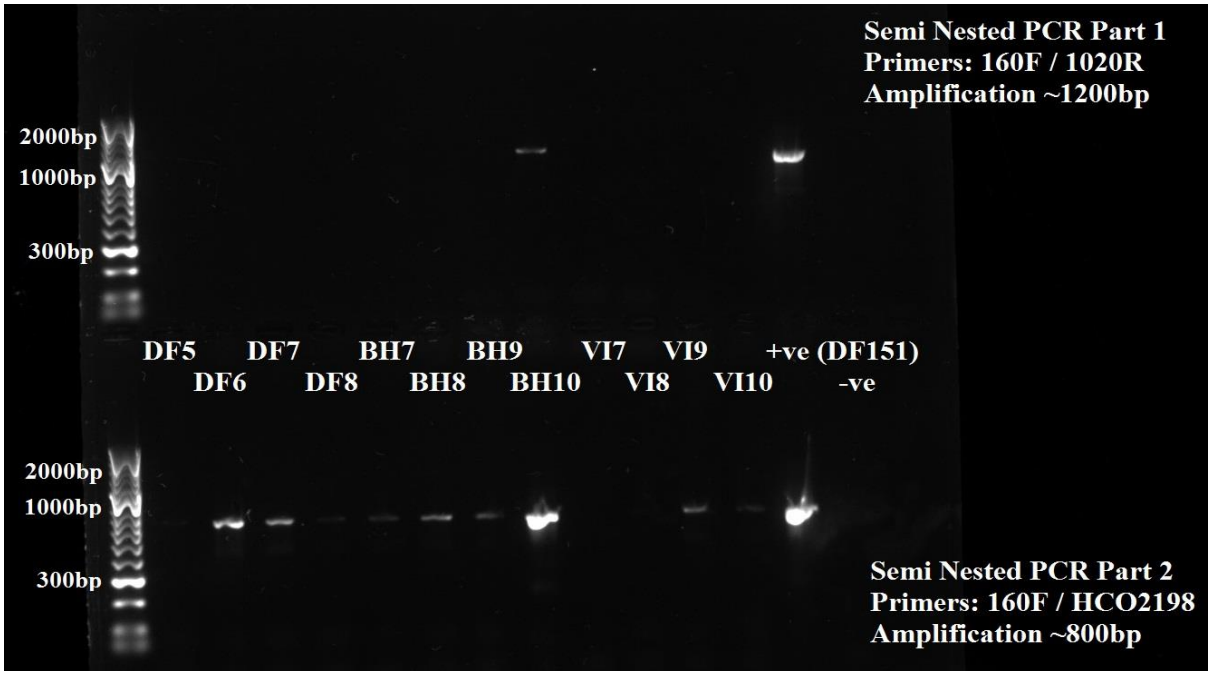


Figure 5.4. Semi-nested PCR of mitochondrial CO1 in *D. farinae* illustrated an observed fragment size of approximately 1200bp and 800bp, respectively, with amplification of samples DF6, DF7, DF8, BH7, BH8, BH9, BH10, VI9, VI10, and the positive control DF151.

As there were varying levels of intensity with this semi-nested PCR (see Figure 5.4), sample VI10 was replicated for the second part of the semi-nested PCR using 6µL of PCR product. This was to ensure that this was not a result of contamination and there was a significant amount of DNA for this sample, which was confirmed in Figure 5.5.

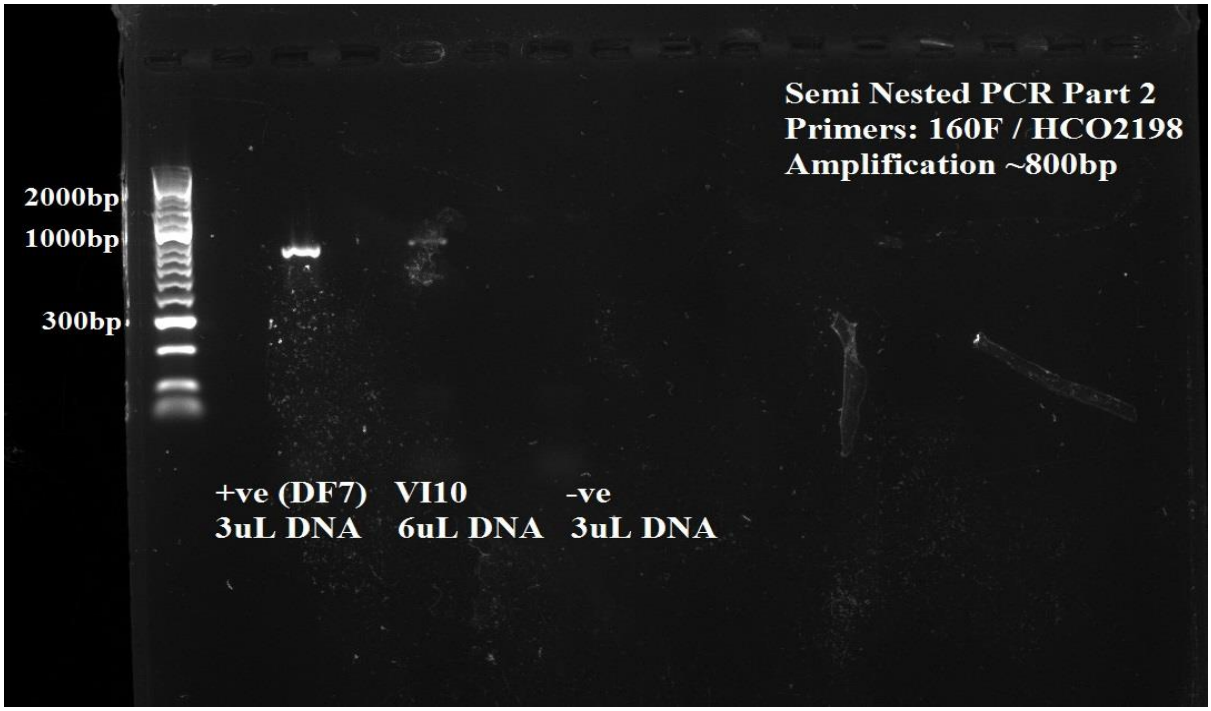


Figure 5.5. Semi-nested PCR part 2 of mitochondrial CO1 in *D. farinae* illustrated an observed fragment size of approximately 800bp for sample VI10 and the positive control, with higher intensity than previously reported.

5.3.2 Nuclear EF1a:

The nuclear EF1a region was amplified using a nested PCR with primers L1/R4 and L4/R1. Only some of the samples which had previously amplified were included (DF6, DF7, DF8, BH7, BH8, BH9, BH10, VI9, and VI10). Samples which did not amplify mitochondrial sequences likely did not have a high enough concentration of nuclear DNA. However, despite the high rate of success of the CO1 amplification, only samples from the DF population showed any amplification during PCR with EF1a primers. No samples from the BH and VI populations amplified (Figure 5.6).

Figure 5.6. Nested PCR of nuclear EF1a in *D. farinae* illustrated an observed fragment size of approximately 800bp and 500bp, respectively, with amplification of DF7, DF8, and the positive control DF151. Despite previous CO1 amplification, only samples from one of the three populations amplified.

5.3.3 Two non-coding regions:

Primers were designed for two non-coding regions of the online published scaffold sequences: 551 L1 / 551 R1, and 101 L1 / 101 R1. These samples were amplified during a single PCR reaction, however once again only samples from the DF population amplified (Figure 5.7).

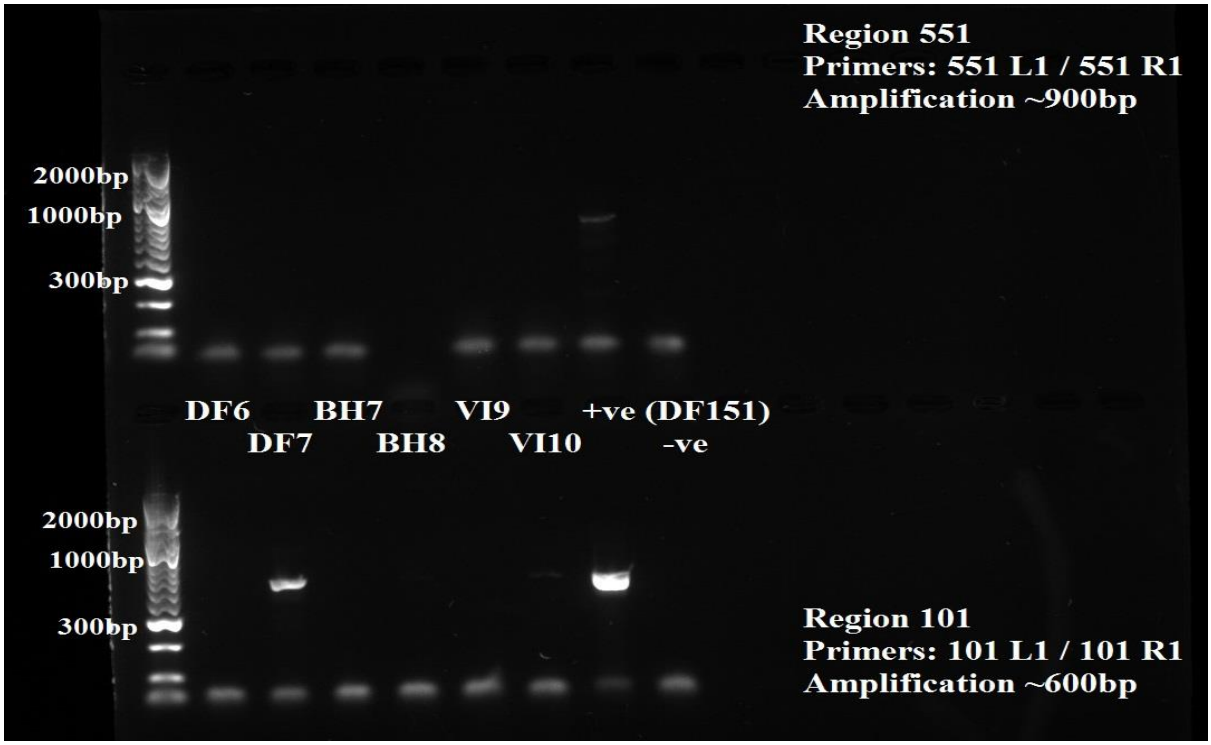


Figure 5.7. PCR of two non-coding regions (551 and 101) in *D. farinae* illustrated an observed fragment size of approximately 800bp and 500bp, respectively. Amplification was observed in the positive control DF151 for region 551, and for DF7 and DF151 for region 101. Only samples from one of three populations amplified.

Although all three populations had samples which amplified for the CO1 region, only mites from the DF population amplified for the EF1a region as well as for the primers designed from the online non-coding scaffold regions. It is possible that the PCR failure may have been caused by differences between the populations which affected the ability for the primers to bind successfully. If the DF sequences were more closely related to the online mite sequence from which the primers were designed, the primers would accurately bind and amplify. However, if the VI and BH populations were more diverged and there were nucleotide polymorphisms, then the primers may have been unable to bind and amplify the region of interest.

5.3.4 Bioinformatics analysis:

To further test this possibility, all amplified PCR products were purified, sequenced, and visualised using the bioinformatics software program Geneious v6.1.8, which allowed alignment between the PCR amplicons and the corresponding online GenBank genome sequence(s). For the CO1 region, the following sequences were aligned: online CO1 Thailand, online CO1 China, online CO1 USA, DF1, DF2, DF6, DF7, DF8, DF9, DF151, BH2, BH9, BH10, VI9, and VI10. For the EF1a region, the following three sequences were aligned: online EF1a USA, DF7, and DF8. Only three sequences were aligned for the 101 scaffold region: online genome China, DF151, and DF7.

5.3.5 *Dermatophagoides farinae* CO1 barcoding:

a. CO1 sequence alignment:

A gene tree for five populations (Thailand, USA, Poland, The Netherlands, and unknown European) with 15 sequences from *D. farinae* CO1 region was created (Figure 5.8). Identical sequences are grouped together, and branch lengths are labelled with the number of nucleotide changes. The online CO1 China sequence (GenBank accession HQ287786) (Yang et al., 2011) was omitted due to an inability to align with the other sequences. This sequence was very short (377bp), and may therefore lack the necessary consensus with the other sequences. There was 95% similarity to the online CO1 USA sequence (GenBank accession GQ465336) (refer back to Table 4.1) (Klimov & O'Connor, 2009). At 95% similarity, the sequences should have able to align, so there may be a contaminant included in the sequencing, or that this CO1 sequence has been misidentified and is not a *D. farinae* mite, but instead a closely related species.

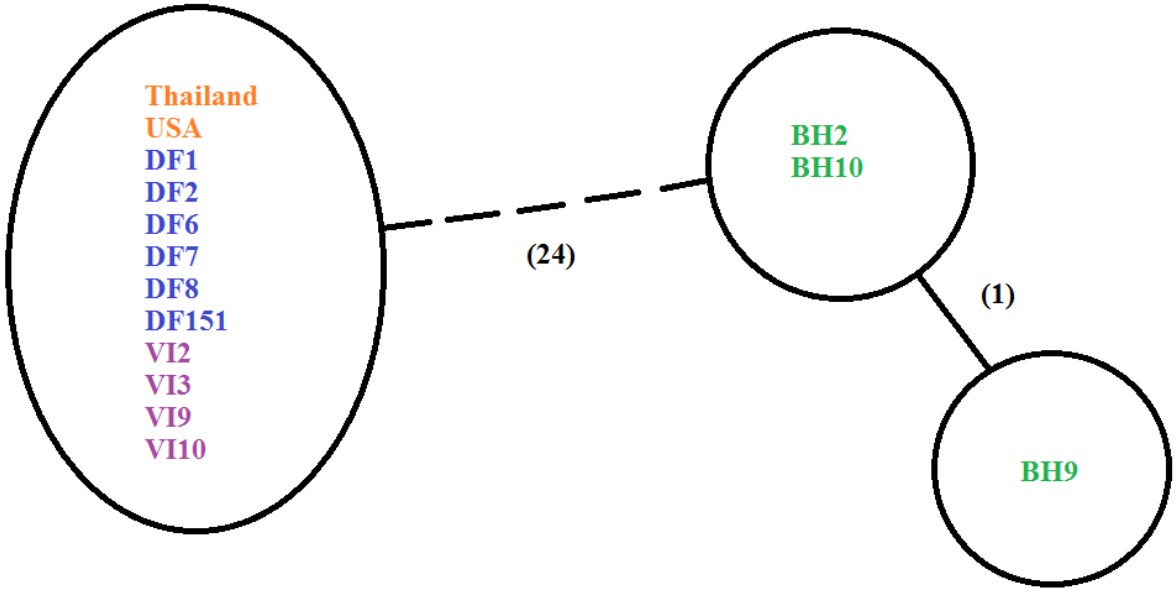


Figure 5.8. Gene tree of nucleotide changes (given in the brackets) in the CO1 gene in 15 sequences from five populations of *D. farinae*. The tree follows the protocol used in PopART, where only the alignments with all nucleotides (511bp) present over the 15 sequences were included (French et al., 2013). Different colours represent the different populations (online, DF, VI, and BH).

In my analysis of the alignments in DnaSP, estimated genetic variation assessed by the degree of polymorphisms within a population were provided as follows: nucleotide diversity π = 0.01633; θ from Eta = 0.01502; and Tajima's D = 0.36451 ($P>0.10$, not significant). These values and Figure 5.8 were supported by an analysis using PopART (http://popart.otago.ac.nz) (French et al., 2013). The number of nucleotide differences at a given site between two DNA sequences (π), and the number of sites where there is a polymorphism (θ), are compared (Moriyama & Powell, 1996). Tajima's D is a measurement of the difference between the two values (Moriyama & Powell, 1996). The majority of changes occurred between sequences from the BH population (The Netherlands), and all other aligned CO1 sequences.

b. DNA barcoding:

The three sequences from the BH population have 24 nucleotide changes out of 606 from the consensus sequence (3.96%). The standard sequence threshold value for barcoding is 10x the mean intraspecific variation (Hebert et al., 2004). Through the examination of North American bird species, an average intraspecific variation of 0.27% was determined, and by applying the 10x principle, a 2.7% threshold for the CO1 gene was established. Therefore, this value of 3.96% is considered larger than would be expected as a measure for intraspecific variation at the CO1 gene.

Although this threshold is useful as a screening technique, it would not be effective on species with short evolutionary histories and recent hybridization (Hebert et al., 2004). It is highly unlikely that a single CO1 barcoding threshold could be applicable across all species, including new animals and geographical locations. However, if a new genomics database were to be developed alongside advancement of CO1 profiling, a comprehensive CO1 database could be designed as a global bioidentification system for animals. This would assist with issues regarding morphological identification to categorise species by recognising sister species, identifying all life stages, quantifying intraspecies diversity, and by making taxonomic decisions objective (Hebert et al., 2003).

DNA barcoding of CO1 regions may provide an indication that despite morphological identifications, distinct populations are actually members of the same species (Hebert et al., 2004). However, if the CO1 sequences are highly divergent, this could be an indication of hidden species diversity (Hebert et al., 2004). As the CO1 sequence divergence was higher than the 2.7% threshold, barcoding scientists would consider the BH population to be an entirely different species than *D. farinae*. This could mean that *D. farinae* mite populations are much

more highly diverse than originally thought, or that samples from this population had been contaminated or misidentified. Therefore, the amplified sequences for samples BH2, BH9 and BH10 were run through the NCBI nucleotide database to establish the top sequence match. All three sequences gave a 99% match to unpublished *D. farinae* sequences from a different CO1 region from China (Zhao et al., 2016).

c. Phylogeny

A phylogeny of 16 *D. farinae* CO1 sequences, as well as one sequence each from *D. pteronyssinus*, *E. maynei*, *A. siro* and *T. putrescentiae* was created to illustrate the divergence between *D. farinae* populations (Refer back to Section 5.2.6). All samples taken during laboratory analysis were included, however, it is noted that some sequences are identical and therefore cannot be completely resolved in the phylogeny. Figure 5.9 used *A. siro* and *T. putrescentiae* as outgroups, as these are commonly identified storage mites, as well as the two other common species of HDMs. This phylogeny supports the high divergence of the BH population identified in the gene tree provided in Figure 5.8, as well as the 3.96% DNA barcoding score. Therefore, it is likely that this population is a cryptic species and has been misidentified.

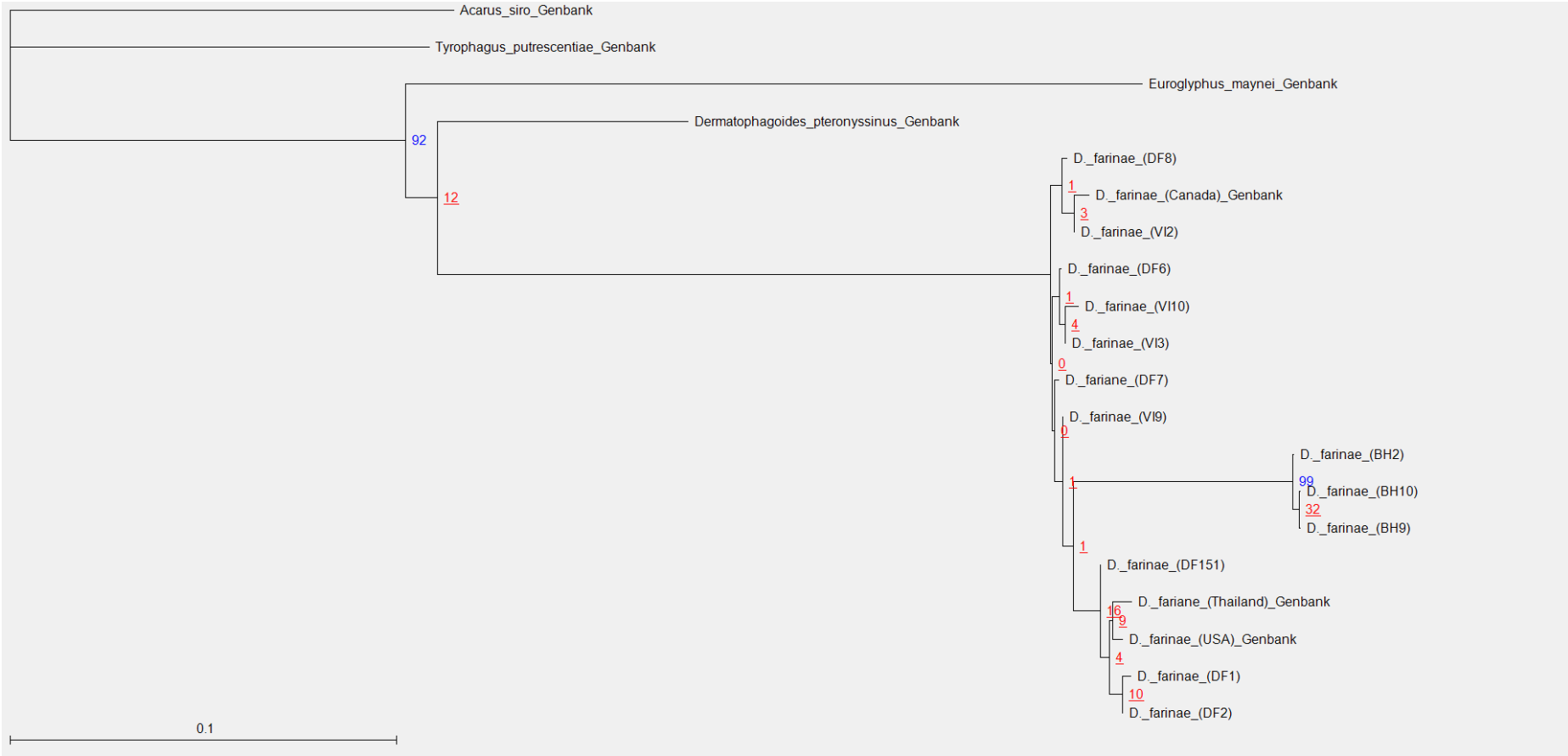


Figure 5.9. Phylogeny of relatedness of the CO1 gene in 16 sequences from six populations of *D. farinae* (DF, VI, BH, online Canada, online USA, online Thailand). The tree includes four sequences, one each from *D. pteronyssinus*, *E. maynei*, *A. siro* and *T. putrescentiae*. This phylogeny illustrates that the BH population has likely been misidentified in previous research and is a cryptic species.

5.3.6 Cryptic species:

a. Identification of cryptic species:

It is not uncommon for a molecular technique to identify cryptic species – species which had previously been morphologically identified as the same species (Rubinoff & Sperling, 2004). Principal component analyses (PCAs) are often employed to confirm the separation of two species, and allow visualisation of dispersal between individuals within a group (Deunff et al., 2004). Size and shape variables can be examined through PCA to separate one cryptic species into formal identification of two species (Klimov et al., 2004). Identifying cryptic species is not only important for taxonomy, but also to assist with understanding speciation, biodiversity, phylogeography, evolutionary theory, and ecological interactions (Skoracka et al., 2012).

b. Cryptic species in mites:

Within the Acari, cryptic species have been identified in the following species: *Spinturnix bechsteini* (parasitic mite) (Deunff et al., 2004), *Cheletophytes venator* and *Cheletophytes torridae* (mites associated with two species of large African carpenter bees) (Klimov et al., 2006), *Cordylochernes scorpioides* (neotropical pseudoscorpion) (Wilcox et al., 1997), *Typhlodromus phialatus* and *Typhlodromus exhilarates* (highly morphologically similar) (Tixier et al., 2006), *Aceria tosichella* (wheat curl mite) (Skoracka et al., 2012, Szydło et al., 2015), *Glaucalges tytonis* (feather mite) (Dabert et al., 2008), and *Sancassania salasi* and *Sancassania ochoai* (Klimov et al., 2004).

It is often necessary to distinguish between mites for successful implantation of biological control, however this can be a very difficult task when there are such small morphological differences between species (Tixier et al., 2006). If slight morphological differences go

undetected, particularly if all populations from various geographic locations morphologically overlapped, then multiple species can be considered a single species for many years (ie *A. tosicella*) (Skoracka et al., 2012). Morphological features may not have had a chance to evolve if lineage separation is very recent (Skoracka et al., 2012).

More cryptic species may be identified in future once more research has been conducted into the hosts' isolation which promotes the appearance of new species (Deunff et al., 2004). To successfully differentiate cryptic species, such as in multi-host feather mite species, both morphological and DNA barcoding analyses are necessary, and assist in speeding up the process (Dabert et al., 2008). As illustrated by *A. tosicella*, speciation is not always reflected by a clear morphological change, and often examination of mitochondrial and nuclear markers between populations is necessary to suggest the presence of a cryptic species with genetically separated lineages (Skoracka et al., 2012).

Compared to sequences from other populations, there was a high level of nucleotide diversity in the BH populations. Although these sequences aligned with unpublished *D. farinae* results from China (GenBank accession KX211990), these populations may be a cryptic species that has been misidentified in the Chinese and BH population. Further morphological and molecular research would be necessary to confirm.

5.3.7 Amplification protocol:

When CO1 semi-nested primers were designed for *D. farinae* samples, the first amplification involved two designed primers, whereas the second amplification introduced the reverse universal mitochondrial primer HCO2198 (Folmer et al., 1994). These three primers did not exhibit self-complementation, binding to each other, or amplification of other species besides

D. farinae when assessed in NCBI. However, if the sequences in Table 5.1 and Figure 5.1a are compared, the HCO2198 primer does not demonstrate 100% sequence similarity with the online CO1 region which was used to design the other primers. Although this does not explain the high level of variation between the CO1 sequences from the BH population and the other populations, it may explain why not all samples amplified during PCR. If there were mutations at additional nucleotides in the primer binding site of some *D. farinae* mites, the combination of this and the full lack of complementarity, the HCO2198 may have been unable to bind. As such, the sample would not have amplified.

With regards to the nuclear regions (EF1a and non-coding regions), only DF mite samples amplified during PCR, and there was no amplification from the BH or VI populations. This is despite earlier amplification with mitochondrial primers. As primers were designed from the online sequences, there is a possibility that these sequences were a closer match to the DF population than the other two populations. If the BH and VI populations were highly diverged from the online sequences, the primers would not have been able to bind and there would have been no amplification of these *D. farinae* samples. Additionally, *D. farinae* could be more than one species – morphologically similar and therefore misidentified by previous scientists. As such, the nuclear primers may not have been specific enough to amplify for these samples, since it was a different species.

5.4 Conclusions

The results from Chapter 5 confirm that due to the low DNA yield obtained from *D. farinae* extractions in Chapter 4, PCR amplification of multi-copy target sequences (CO1 mitochondrial) was more successful than amplification of single-copy target sequences (EF1a and non-coding regions). Analysis of the mitochondrial CO1 gene in *D. farinae* illustrated a high level of diversity between 15 sequences from five populations of European, North American, and Asian origins. There was 3.8% diversity between the BH population (The Netherlands) and the other populations, with a high level of intraspecific mitochondrial DNA divergence indicating that a cryptic species may exist. Previous results in other mite species support the possibility that samples of *D. farinae* have been misidentified in the past and are two separate species.

The CO1 universal primer (HCO2198) did not illustrate 100% sequence similarity with the examined *D. farinae* sequence. Therefore, primers should be developed from the published genome, and universal primers discarded to ensure high levels of amplification. Further research would need to be conducted into mitochondrial and nuclear genes involving more *D. farinae* populations worldwide would be necessary to corroborate these preliminary findings.

Chapter 6: Exploring Costs and Benefits of Different Mating Systems

6.1 Introduction

The previous chapters have illustrated that there are still deficits in our knowledge of mites. There is very little information available regarding the genetic systems of a lot of species, however it has been shown that mites have a wide variety of reproductive mechanisms (refer to Chapter 1, Section 1.4, and Table 1.2). As such, mites are an important taxon to act as a model for mating system evolution. One of the main focuses of my research in Chapters 6 and 7 is to examine haplodiploidy, a known mating system in mites.

This chapter seeks to enhance knowledge of genetic systems by examining different ploidy systems. Between organisms, ploidy level varies from haploid (one chromosome set), diploid (two chromosome sets) and polyploid (more than two sets of chromosomes) (Gerstein & Otto, 2009) (Figure 6.1). Models are useful because they mimic the individual in question, however, the researcher is able to set parameters to control different factors that they would not be able to control for in the wild. The system is easily manipulable, and it is easy to determine whether a certain aspect is the most important. As this is a theoretical approach, it can provide support to prior experimental results, or suggest a new theory which can be explored in the lab. Much of the previous research into haplodiploidy in mites has focused on the spider mite, *T. urticae*. This research has shown that in the haplodiploid spider mite, sex ratio is controlled by each sex to their own advantage; this often occurs by manipulating egg size in females, as larger eggs will have an increased chance of fertilization (Macke et al., 2012, Macke et al., 2014). In addition, the host plant on which the population evolved had an effect on sex ratio, with females on cucumber plants producing a higher proportion of males when compared to females on

tomato plants (Marinosci et al., 2015). Spider mites are able to adapt to different host plants, and their long-term evolution on one type of plant does not prevent them from adapting to a different environment (Magalhães et al., 2007).

In Chapter 6, firstly, some organisms with interesting and different mating systems (*Saccharomyces cerevisiae*, and *Ulva*) will be examined, before discussing mating systems commonly identified in mites (diploidy and haplodiploidy). It is necessary to examine the advantages and disadvantages of being a haploid or a diploid individual first, to understand what benefits and detriments would be encountered by being a haplodiploid. Although this is sufficient to explain the existence of an individual in one state or another, some alternative theories regarding ploidy influence will be explored. Finally, through computer simulations, different models will be tested to expand our understanding of the consequences of evolutionary factors, such as selection and mutation, on a variety of mating systems. Ultimately, high divergence from the ancestor over time is positive for the individual if a mutation is beneficial, however it is bad for the individual if a mutation is deleterious.

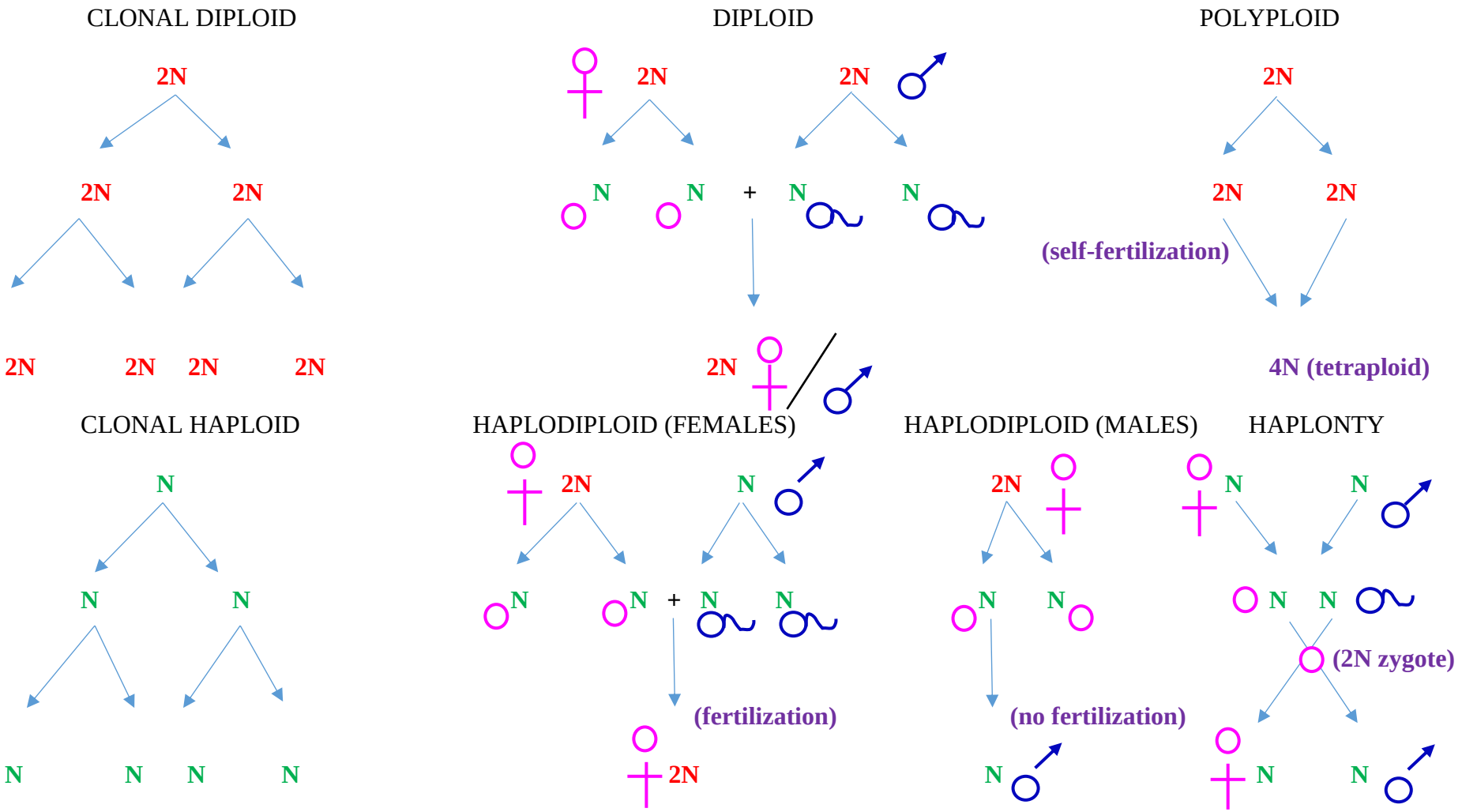


Figure 6.1. Schematic of five mating systems. Representatives of haploids include bacteria, some fungi, some protists, and moss, whereas most vertebrates, ferns, and seed plants are diploid (Gerstein & Otto, 2009). Polyploidy is less common in animals, but identified quite often in a variety of plant species (Gerstein & Otto, 2009). Mites are known to reproduce through diploidy, thelytoky, arrhenotoky, and haplodiploidy (see Table 1.2); therefore, this represents some different systems explaining the usefulness of mites as model organisms for mating system evolution.

6.1.1 Experimentation in yeasts:

a. The use of *Saccharomyces cerevisiae*:

The model organism, *Saccharomyces cerevisiae*, is advantageous in ploidy research, and therefore can be used to empirically measure differences in ploidy. The primary state of *S. cerevisiae* is diploid, meaning it differs from the many other yeast species which are haploid, such as *Kluyveromyces lactis*, *Saccharomyces pombe*, and the methylotrophic yeasts (obtain carbon and energy for growth solely from methanol) (Dujon, 2010). Although some organisms are haplontic (life cycle is haploid with a brief diploid zygote phase), *S. cerevisiae* is diplontic, meaning all life cycle stages with the exception of gametes are diploid (Hanson & Wolfe, 2017). Figure 6.2 illustrates the life cycle of *S. cerevisiae.*

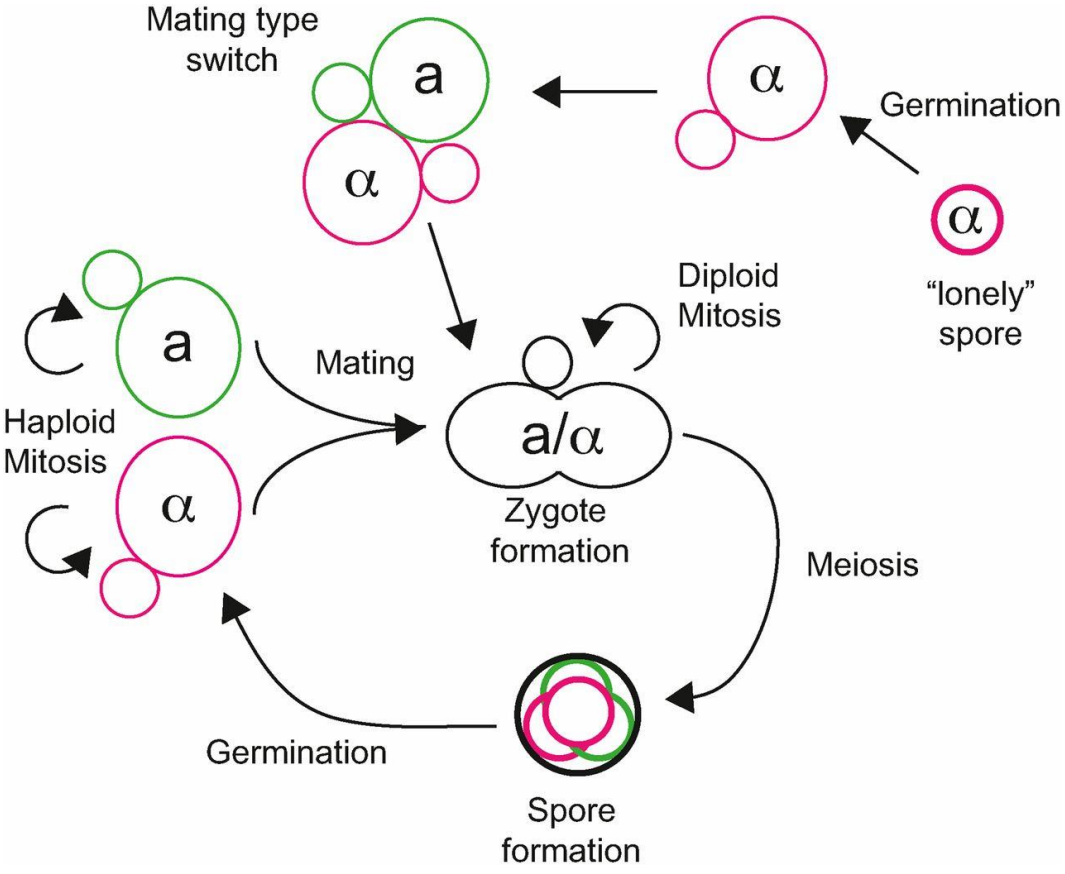


Figure 6.2. Life cycle of haploid and diploid mating types of *S. cerevisiae*, with cells in either the **a**, α, or **a**/α form (Hanson & Wolfe, 2017).

b. Yeast mating-type switching:

In *S. cerevisiae*, cells come in one of three forms: **a**, α, and **a**/α, and differ in DNA content at the *MAT* locus which specifies cell type (Hanson & Wolfe, 2017). In most organisms' cell type is irreversible, however mating (cell)-type switching can occur between the haploid states (**a** and α) by changing genotype at the *MAT* locus from *MAT***a** to *MAT*α, and vice versa. This process is tightly regulated to ensure switching occurs only when the outcome will be successful mating (Hanson & Wolfe, 2017), and is a rapid process, occurring within a single cell division (Haber, 2012). This process (homothallism) means that the subsequent mating with the opposite type allows for self-diploidization. The *MAT***a**/*MAT*α diploids are non-mating, and provide the yeast with advantages such as meiosis, and spore formation in nutritionally poor environments (Haber, 2012). All three types of *S. cerevisiae* cells divide mitotically under favourable environmental conditions (Hanson & Wolfe, 2017). Unlike some other species, nutrient availability has no influence, and the haploid species undergoes both mating and mating-type switching even in nutrient-rich environments (Hanson & Wolfe, 2017).

c. Exposure of *Saccharomyces cerevisiae* to varying environments:

S. cerevisiae ploidy is able to shift to suit the environment for immediate fitness increases; however ideal environments for each ploidy levels have yet to be discovered (Gerstein & Otto, 2009). Haploids and tetraploids are prone to evolve to be diploids (Gerstein et al., 2006, Mable & Otto, 2001). This is potentially due to *S. cerevisiae* haploids and tetraploids trying to revert back to their typically diploid state, or diploidy simply happens to be the most stable state for this yeast species (Mable & Otto, 2001). Table 6.1 provides some examples of how haploid, diploid, and tetraploid *S. cerevisiae* adapt to varying environments.

Table 6.1. Adaptation of different mating systems in a variety of *S. cerevisiae* strains provides a summary of how changing environments can result in different mating system successes.

States	Conditions	Mating System Success	Additional Comments	Reference
Isogenic haploids, and diploids	Carbon-limited chemostat environment	Adaptive rate of change 1.6x higher in diploids		(Paquin & Adams, 1983)
Two strains: haploids, diploids, and tetraploids	DNA-damaging agent ethane methyl sulphonate (EMS)	Reduction in haploid population size compared to diploid and tetraploid populations	Inability of haploids to mask deleterious mutations	(Mable & Otto, 2001)
		Equal growth reduction rates between diploids and tetraploids	Higher ploidy may not have a significant impact on a cells ability to mask deleterious mutations	
		Similar increase in growth rate between haploids and diploids	Potentially caused by severely deleterious mutations immediately eliminated in haploids, but there is a delay in the removal of mildly deleterious mutations	
			Results may have been influenced by toxic effects of EMS reducing fitness (instead of direct mutations), as well as changes towards diploidy making it difficult to determine to what degree growth and masking were dependent on the original ploidy	
Haploids, and diploids	Large population size	Haploids adapt faster than diploids	10 populations over 2000 generations	(Zeyl et al., 2003)
	Small population size	Diploids adapt faster than haploids		
Haploids, and diploids	Antifungal drug fluconazole (high concentrations)	Haploids adapt faster than diploids	Recessive mutations in *ERG3* causing resistance to be favoured	(Anderson et al., 2004)
	Antifungal drug fluconazole (low concentrations)	Diploids adapt faster than haploids	Mutations in *PDR1* and *PDR3* genes (regulate transporters that confer resistance to fluconazole)	

			Genes are favoured at low concentrations, therefore mutations quickly become fixed in the population	
Isogenic haploids, diploids, and tetraploids	Salt-stressed media (long-term study)	Haploids converge to diploids	1766 asexual generations	(Gerstein et al., 2006)
	Unstressed media	Tetraploids converge to diploids		
Haploids, diploids, and tetraploids	YPD (yeast extract peptone dextrose) + salt	Haploids adapt faster than diploids or tetraploids		(Gerstein & Otto, 2009)
		Diploids spread through haploid populations faster than normal under standard YPD conditions		
Haploids, and diploids	YPD / YPD + salt (short-term study)	Haploids adapt faster than diploids	7 environments for 200 generations Results true for all seven environments, with three significant	(Gerstein et al., 2011)

6.1.2 Reproductive strategies in algae:

Species of the sea lettuce, *Ulva*, reproduces similarly to *S. cerevisiae*, by alternating haploid and diploid cycles of reproduction (haplodiplonic) (Wichard et al., 2015). Diploid sporophytes arise from the fusion of two cells which mate with the opposite type, and haploid cells originate from meiotically-formed haploid cells or unmated biflagellated gametes (Wichard et al., 2015). In mosses, the haploid stage is significantly larger than the diploid stage, whereas in ferns, the reverse is true (Bell, 1997). The life cycle of *Ulva* as a representation of plant and algae reproduction is given in Figure 6.3.

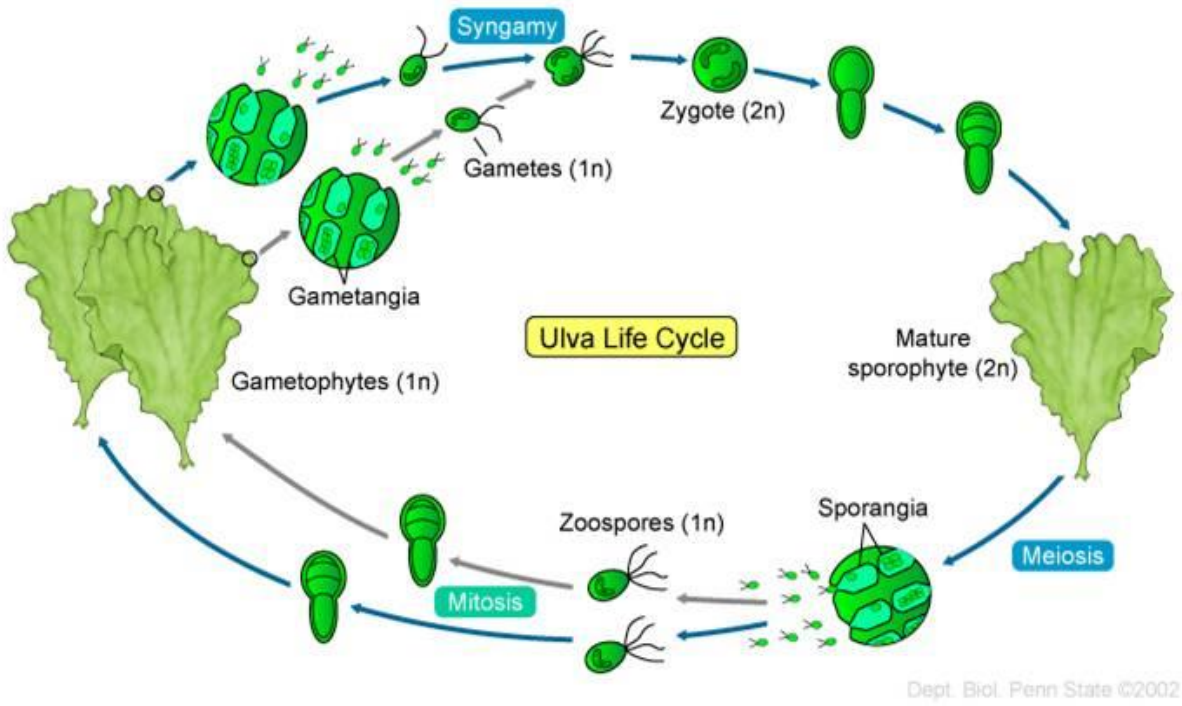


Figure 6.3. Life cycle of the sea lettuce, *Ulva*, illustrating alternating haploid and diploid cycles (2013). This provides an example of an uncommon mating system.

A 1:1 sex ratio is most common in algae, however if this is not the case, female gameotophytes are more frequent (De Wreede & Kinger, 1988). The biphasic life history can either be isomorphic (*Ulva*), where the sporophyte and gametophyte are similar in appearance, or heteromorphic (kelps), where either the sporophyte or gametophyte can be larger. A triphasic

life history also exists for the red algae, where there is an additional diploid spore-producing phase. As in *S. cerevisiae*, diploidy is the dominant state of algae. Although parthenogenesis frequently occurs with members of the algae in laboratory culture, further study in wild populations is necessary (De Wreede & Kinger, 1988).

6.1.3 Prevalence of diploidy:

a. Increased beneficial mutations:

There is a bias towards diploidy in many organisms, with nearly all metazoan taxa undergoing somatic development in this state (Mable & Otto, 1998). It has been hypothesized that diploids evolve at a faster rate than haploids due to having twice as many alleles, which also makes diploids more likely to carry a new beneficial mutation. However, this does not necessarily mean beneficial mutations will evolve at a faster rate than in haploids (Mable & Otto, 1998). The rate at which a population adapts to a new environment is dependent on the rate of fixation of beneficial mutations (Gerstein et al., 2011). In diploids, there may be slower adaptive evolutionary increase if fitness benefits are masked by the alternative allele (Mable & Otto, 1998). There is an assumption that advantageous mutations are dominant or semi-dominant (therefore expressed in the heterozygous state), whereas deleterious mutations are recessive (Paquin & Adams, 1983). As such, if the beneficial mutation is dominant and genetic mixing is frequent, diploidy will be favoured over haploidy (Mable & Otto, 1998). But if the mutation is rare, it will be lost more easily from the diploid population (Mable & Otto, 1998, Orr & Otto, 1994).

b. Two copies of genes:

Diploids have the ability to expand their genome at a faster rate than haploids due to insertion of both new and old genes simultaneously (Lewis & Wolpert, 1979). For a new beneficial gene to be created, the present gene must first be mutated; however it is often necessary for the original gene to remain to carry out its current function. In diploids, there is a "spare copy" of any given gene automatically present, whereas in haploids, there is only one copy of the gene; therefore duplication must occur prior to mutation. In the diploid genome, new beneficial mutations can be taken to fixation, as well as being integrated and sexually recombined to add diversity and preserve this state (Lewis & Wolpert, 1979). Ultimately, in a sexual population, multiple beneficial mutations can be incorporated into an individual genome at the same time through recombination; whereas in an asexual population, multiple mutations can only be added sequentially once the first mutation has occurred (Crow & Kimura, 1965).

c. Masking deleterious mutations:

The overwhelming advantage to being a sexual diploid is masking of deleterious mutations within a genome (Mable & Otto, 1998). Deleterious mutant alleles are rare within a population; therefore it is unlikely for a diploid to possess two mutant alleles at the same locus. In contrast, haploids will express every allele at a locus and only one mutant copy is required for a decrease in fitness. Deleterious mutations persist longer in a diploid population, compared to the immediate exposure and removal in haploids. Consequently, genetic variation is higher in diploid populations (Mable & Otto, 1998). Increased genetic variation would be advantageous to diploids if these deleterious recessive mutations were to abruptly become beneficial in a novel environment, resulting in quicker adaptation to the changing environment (Otto & Gerstein, 2008).

6.1.4 Prevalence of haploidy – fewer mutations:

When deleterious mutations are introduced, the mean fitness of a population depends mainly on the genome-wide deleterious mutation rate and not on the selective disadvantage of the mutations (Haldane, 1937). These deleterious mutations must then be removed by selection. Mutations are "masked" for a period of time in diploids and polyploids, and therefore they are present in the population for more generations and reach higher frequencies before being eliminated (Haldane, 1937). In haploids, deleterious mutations are immediately exposed and removed, therefore those individuals that survive are of a higher fitness and carry fewer mutations than a diploid (Mable & Otto, 1998). Any offspring will subsequently have fewer mutations and higher fitness, with selection efficiently removing any future deleterious mutations (Mable & Otto, 1998).

6.1.5 Why be both haploid and diploid:

a. Advantageous balance:

Although both extended haploid and extended diploid phases should be favoured under certain conditions, individuals tend to favour either diplonty or haplonty (Mable & Otto, 1998). Changes in ploidy level, such as alternating between haploid and diploids phases in higher plants, may be brought on by environmental changes (Ewing, 1977). Often generations are unequal and the organism exists in one state for most of the time, but undergoes reproduction in the other phase. When selection acts at both haploid and diploid phases of development, the retention of variability in the population can differ. The occurrence of a single diploid phase within a mainly haploid organism helps maintain a heterozygous advantage and frequency-dependent selection in the population. In contrast, this equilibrium could not be maintained over time in only haploid populations (Ewing, 1977).

In some instances, both phases are maintained (haplodiploidy) (see Figure 6.3). Therefore, even though haplodiploidy may seem like an unusual system, many organisms have both haploid and diploid stages. The amount of time spent in each stage is variable, but this indicates that perhaps the system is not as strange as it initially appears. This provides the organism with two different methods of dispersing genetic material, increases exploitation of resources, and sex occurs half as often (useful in conditions where costs of sex are high) (Mable & Otto, 1998). Generally, diploids will have higher fitness if the only form of evolutionary change is mutation, whereas haploids will have the advantage if selection is the only evolutionary force (Otto & Gerstein, 2008). This is based on both beneficial and deleterious mutations being immediately expressed in haploids, so selection can "see" fitness effects of mutations more easily. Deleterious mutations are immediately removed and reach lower equilibrium frequencies, and beneficial mutations easily spread through the population (Otto & Gerstein, 2008).

b. Rates of adaptation:

The rate of adaptation is influenced by both the time it takes for mutations to appear, as well as how long it takes for the mutation to be fixed at a high frequency in a population due to directional selection (Orr & Otto, 1994). Although diploids have increased fitness due to double the number of alleles to be beneficially mutated, they will only profit if mutations are dominant (and disadvantageous if recessive), and there is no effect to the rate of adaptation if mutations are additive (genes do not interact and their effect is the sum of each mutation) (Zeyl et al., 2003, Manuel Pérez-Pérez et al., 2009, Orr & Otto, 1994). In the presence of recessive mutations, haploids will respond more efficiently to selection (Orr & Otto, 1994). The rate of fixation of adaptive mutations is dependent on population size, therefore producing the larger diploid cells may come at an increased cost (Paquin & Adams, 1983). The effects of mutations on three different mating systems is given in Table 6.2.

Table 6.2. Comparison of beneficial and deleterious mutations, as well as the expected rate of adaptation in haploid and diploid populations, based on previous publications, as well as the predicted outcomes with haplodiploid populations.

	Haploid	**Diploid**	**Predicted Haplodiploid**
Beneficial Mutations	Half as many alleles as diploids	Twice as many alleles, therefore twice as many chances for beneficial mutations	Would increase twice as quickly in females (diploid) compared to males (haploid)
	Any gene must first be mutated prior to duplication to keep original function	Spare copy of gene means new beneficial mutations inserted rapidly into genome	Retention of variability in environments due to having two different states
Deleterious Mutations	Immediately expressed and removed	Masked, therefore persist longer in the population	Deleterious mutations immediately exposed and removed in the male haploids, increasing fitness
	Individuals that survive have higher fitness and carry fewer mutations, as will offspring	Advantageous in an individual but inherited by offspring and accumulation is ultimately disadvantageous	
		Increased genetic variation could be useful if deleterious mutations became beneficial in novel environment	
Rate of Adaptation	Faster in large populations (Zeyl et al., 2003)	Fixed more often (Paquin & Adams, 1983)	If mutations dominant, diploids have increased fitness
	Faster in short term yeast studies (Gerstein et al., 2011)	Faster in long term yeast studies (Gerstein et al., 2006)	If mutations recessive, haploids respond better to selection

c. Maternal and paternal selection levels:

In sexually reproducing organisms, both a haploid (postmeiotic) and diploid (postsyngamy) phase exist, however selection does not necessarily occur in both phases (Otto et al., 2015). Instead diploid parents can modify the level of selection during the haploid phase to suit their needs. In the models designed by Otto *et al*. (2015), the organism is assumed to be mainly diploid, with genes in the diploid parent affecting the gametic phenotype and evolving the strength of selection in the haploid phase. Fathers evolve competitive sperm to mask mutations in the haploid gametes, thereby acting to reduce the exposure of their gametes to haploid selection prior to fertilization. In contrast, maternal control occurs after fertilization, with haploid selection strengthened by the corresponding genes. If mutations which are positively-selected for in the haploid phase are negatively-selected in the diploid phase, fitness will be decreased in the diploid offspring. As such, females have evolved a high level of gametic selection to counterbalance the occurrence of deleterious mutations (Otto et al., 2015).

It would be expected that in mammals, egg cells are carefully shielded from selection to increase the chances of normal embryonic development and survival. However, research has illustrated that there are a large number of reproductive proteins that show evidence of evolution by positive Darwinian selection (Swanson et al., 2003). As egg coat proteins are subject to positive selection, it was questioned by Swanson *et al*. (2003), whether sperm receptors would also have to change to allow continued compatibility. This analysis could not identify the selective pressures, but did illustrate that the areas subjected to positive selection were in sperm-egg-binding domains (Swanson et al., 2003). This indicated that there was a selective force related to male-female interaction (fertilization). If selection contributed to rapid evolution resulting in a mismatch between the egg-sperm-binding proteins, infertility would

occur, a dire consequence of a positive selection arms race between egg and sperm (Swanson et al., 2003).

6.1.6 Alternative theories regarding ploidy influence:

There have been some alternative theories postulated to explain discrepancies between haploids and diploids. To be thorough to this report, a few hypotheses will be described below, however the research outlined in the previous sections are deemed sufficient to explain the advantages and disadvantages of haploidy, diploidy, and haplodiploidy.

a. Non-genetic factors:

Cavalier-Smith (1978) hypothesized that non-genetic factors, particularly cell size, contribute to differences in ploidy number. Organisms with a larger body size are strongly *K*-selected and will be multicellular, whereas organisms which are strongly *r*-selected are unicellular. The varying degrees of *r*- and *K*-selection leads to ploidy disparities. Additionally, DNA has both structural and genetic purposes, and non-transcribed DNA determines the size of the nucleus, and therefore the size of the cell. Varying levels in haploid DNA content (DNA C-value) respond to selection, which creates variability in cellular and organismic properties. Organisms with low C-values are small celled and reproduce quickly, whereas organisms with high C-values have large cells and grow slowly. Due to the double volume difference between diploid nuclei/cells compared to haploid cells, when large size is selectively advantageous the life cycle will be diploid, and when small size is advantageous the life cycle will be haploid (Cavalier-Smith, 1978).

b. Contradiction to Cavalier-Smith's hypothesis:

Lewis (1985) disputed this theory, with one of the main issues the assumption that all simple organisms which are haploid have been strongly *r*-selected. In actuality, some simple organisms are diploid which violates this principle (Lewis, 1985). I agree with this notion, as there will be instances where haploid genomes have double the volume, meaning the cell will be larger. This large cell size does not necessarily mean it will be diploid. Likewise, some smaller and simpler organisms with reduced cell sizes can be diploid. There is some evidence from plants that evolution to higher ploidy number results in an enlarged cell size (Ranney, 2006). Fruit from tetraploid apples may be twice as large as the fruit from diploid apples; however, this increased size also results in watery and misshapen fruit (Ranney, 2006). Plants cells have the ability to change their shape while maintaining their volume, therefore in some instances the previous would not happen and cell structure would hold (Kondorosi et al., 2000). Advances over the past forty years have likely made this hypothesis less applicable than when it was first suggested.

c. Nutrient-sparing hypothesis:

In the nutrient-sparing hypothesis, haploidy is advantageous based on nutrition rather than genetics, under specific conditions (Lewis, 1985). The retention of haploidy is due to three factors: the relationship between DNA, cell size and mitotic cycle; how selection pressures affect cell size when numerous cells are present; and differences in selection pressures when obtaining nutrients and consuming energy at the same time. If elements such as nitrogen and phosphorus can be accounted for in DNA, there is a reduction in the total element inventory for a fixed cell size. Haploids have a reduced DNA inventory to help with nutrient intake, and due to size differences between haploids and diploids, the nutrient-sparing effect in the former

can save 25-50% of any nutrient. The mixture of ploidy levels found in the algae and fungi is consistent with this hypothesis (Lewis, 1985).

This concept was studied by examining whether species with a large genome size and/or high ploidy level are limited by the availability of nitrogen and phosphorus (Guignard et al., 2016). Genome size widely varies in plants, a factor which influences nutrient demands, and consequently the ability to grow and thrive in a variety of environments. As such, larger genomes may have an increased cost due to the requirements of nitrogen and phosphorus for nucleic acids. Genome size was larger in nutrient rich environments with both nitrogen and phosphorus, whereas on control plots with only nitrogen or only phosphorus added, genome size was significantly smaller. This supported the hypothesis that genome sizes are costly when there is limited nitrogen and phosphorus nutrient availability. The authors suggest that these two nutrients are linked with an organisms DNA requirements (Guignard et al., 2016).

d. Contradiction to Lewis's hypothesis:

The small size of haploid cells results in a higher surface area to volume ratio, as well as half the energetic costs of DNA replication compared to diploids (Mable, 2001). As such, haploidy is advantageous in nutrient poor conditions, and it could be expected that diploids grow better in nutrient rich conditions. However, experiments indicated that haploids performed better than diploids in nutrient rich conditions, and diploids and haploids performed similarly in nutrient-poor conditions. Tetraploids demonstrated poor results all around in comparison to the other ploidy states, and at higher temperatures, haploid cell size and growth rates increased. Despite previous experiments these results do not support the nutrient-sparing hypothesis (Mable, 2001).

6.1.7 Haplodiploidy is expected to show adaptive evolution:

As previously demonstrated in *S. cerevisiae*, haploid, tetraploid, and diploid yeast all tried to maintain life as diploids, indicating that this may be the most stable state, or there is a natural inclination of organisms to try to retain this ancestral form (Mable & Otto, 2001). In addition, mutations do not introduce enough variation into a population, and the process of meiosis (haplonty or diploidy) resulting in a certain level of recombination may be a requirement for development and survival. There are, however, "ancient asexuals", which have survived over extended periods of time without sex and recombination (Schaefer et al., 2006). For example, there are no known males in the bdelloid rotifers, therefore their mating system was determined to be fully asexual reproduction by thelytoky (Pouchkina-Stantcheva et al., 2007). However, see the following for evidence of how this species also uptakes bacterial DNA from the environment (Gladyshev et al., 2008). The lack of sexual recombination makes environmental adaptation difficult, as genetic diversity can only arise through mutations. It was determined that in the rotifers, two LEA proteins, involved in desiccation, diverged in function. One protein maintained its original purpose, whereas the other does not, but can assist with membrane integrity during drying. This provides an example of how asexual reproduction could lead to genetic diversity (Pouchkina-Stantcheva et al., 2007). A commonly deployed test to examine the long-term absence of recombination is the "Meselson effect", which assumes that in a diploid the two allelic copies at a locus will diverge over time as mutations are independently accumulated (Welch & Meselson, 2000). Nevertheless the presence of the Meselson effect is not conclusive of long-term absence of recombination, as low rates of sexual reproduction mimic this effect – something which has also been seen in the bdelloid rotifers (Ceplitis, 2003, Boschetti et al., 2012, Boschetti et al., 2011).

Due to the exposure and removal of deleterious mutations resulting in increased fitness in offspring, haploid individuals have an advantage over diploids. The masking effects in diploids permit deleterious mutations to travel through the population and reduce fitness in future generations, as well as reducing the impact of beneficial mutations. However, diploids thrive from beneficial mutations and having two copies of a gene, suggesting why most organisms are still in this ancestral state. As such, haplodiploidy incurs the advantages of both systems, and therefore has better adaptive evolution compared to individuals which are only diploid. This concept was explored in the models discussed in the remainder of the chapter.

6.2 Materials and Methods

6.2.1 Developing Perl:

The programming language Perl 5 (Schwartz et al., 2017) was used to simulate populations using the following mating systems: haplodiploidy, diploidy, haploidy with recombination, clonal haploid, and clonal diploid (Figure 6.1). A haploid is represented by a string of numbers depicting the accumulative mutations at loci. Diploid individuals have two haploid genomes and can therefore be heterozygous or homozygous for mutations at a particular locus. They are therefore represented by two strings of numbers depicting the accumulative mutations at loci. The number of males and females are set at the beginning and numbers remain constant across generations.

Males are made through a spermatogenesis subroutine, and females are made through an oogenesis subroutine. Mutations are introduced at spermatogenesis and oogenesis, and only non-lethal mutations are considered. New individuals are made by choosing parents from the previous generation, and checking their fitness. Selection is imposed at mate choice. Fitness is checked for all individuals by comparing against the fittest male / female in the population. The fitness effect of mutations are influenced by dominance, where fully dominant mutations in their heterozygous state (01) are equivalent to the effect of a mutant homozygote (11), and fully recessive mutations are equivalent to the effect of a wild type homozygote (00). Dominance degree meant that the absolute fitness of an individual will depend on sex, so fitness relative to others of the same sex is used to decide chances of mating success. If the individual is fit enough, it is allowed to mate. If the individual is not fit enough, it is discarded and a new individual is selected. New individuals are created until the predetermined population size and sex ratio is reached. Through the generations, the accumulation of mutations are monitored, as

these are fixed or lost from the population. Full programs are provided in Appendix 2, Figures 6.10 – 6.14.

The model is stochastic with the following parameters that could be altered (not all are applicable in every mating system): number of genes, census population size, sex ratio (illustrating the proportion of males in the population), number of generations, mutation rate, selection coefficient, and degree of dominance. The number of genes was kept constant at 20 genes, which mutated at a rate of $\mu = 10^{-3}$. In the wild, there would be higher number of genes and a lower mutation rate of approximately 10^{-6} (Bürger & Bagheri, 2008, Lynch, 2010, Lynch, 2011). Matings are modelled between individuals with specific genotypes, with mutations occurring in particular genes to allow for future iterations of the model to include inbreeding. There are separate sexes and a directly manipulable sex ratio, which is imposed on the population, rather than being allowed to evolve.

Three different degrees of dominance (complete dominance (0), co-dominant (0.5), or complete recessiveness (1)) were tested at each stage, at different population sizes and number of generations. Analysis of average dominance across studies conducted in the past forty years (at time of publication) illustrated an average dominance value of 0.27 (confidence interval [0.18, 0.36]) (Manna et al., 2011). In addition, the selection coefficient of new mutations varied from strongly recessive (0.8) to strongly beneficial (1.2), inclusive, representing the fitness disadvantage given to the individual. The simulations were performed to measure the accumulation of mutations, both beneficial and deleterious, over time, and compare this between mating systems. Focus was on altering one of two parameters: sex ratio or selection coefficient. Figure 6.4 provides a representation of how the program is designed for a haplodiploid population.

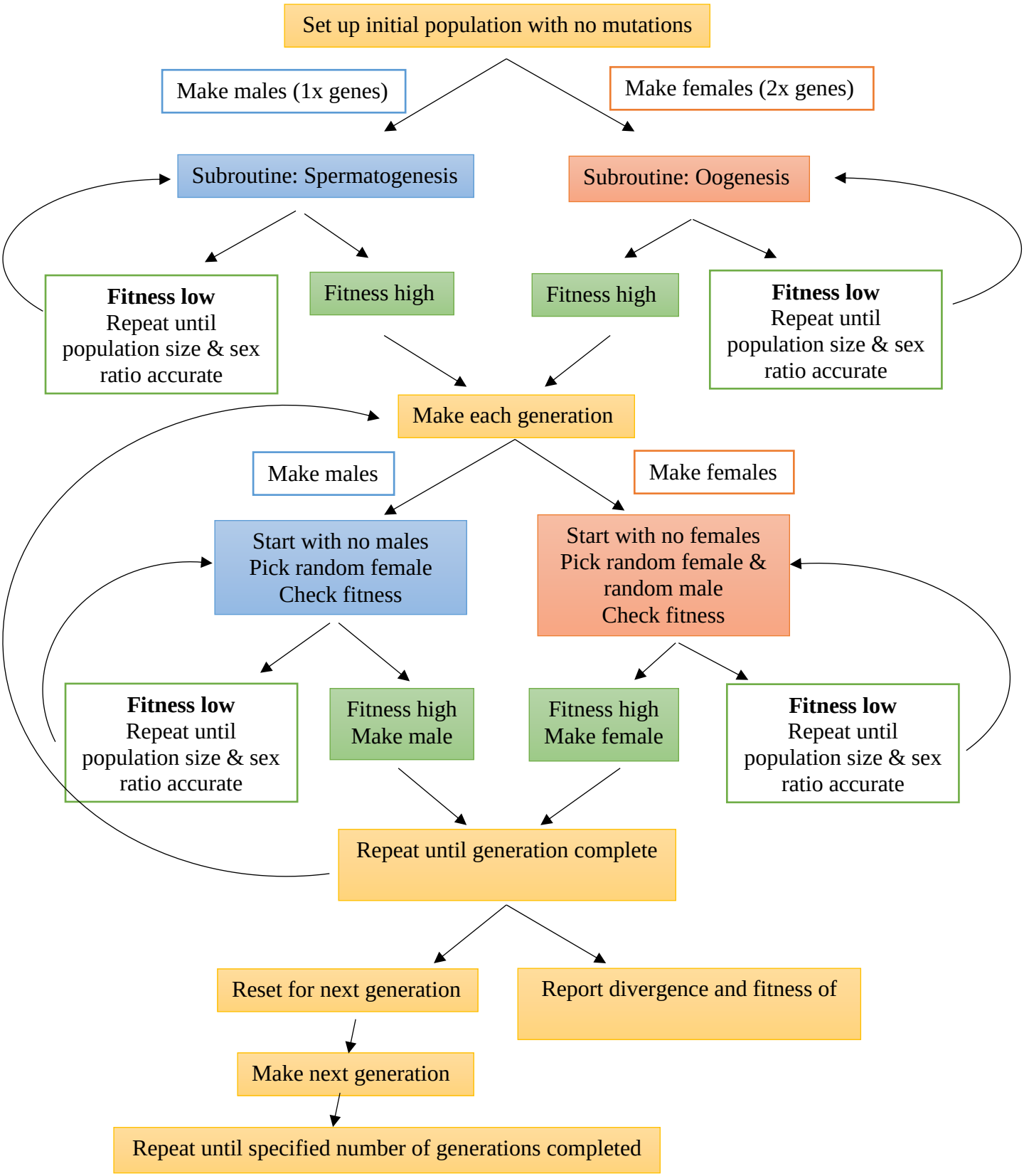


Figure 6.4. Representation of Perl program for a haplodiploid population. Other programs follow a similar format, however with differences in the number of genes and the involvement of male reproduction.

6.2.2 Altering parameters – selection coefficient:

The first set of simulations were performed with a changing selection coefficient between 0.3 – 1.8, while keeping the sex ratio constant at 1:1 (0.5). The selection coefficient is representative of fitness, with a value of 0.8 incurring a 20% fitness disadvantage. When mutations are beneficial, it is useful for the mutation to be accumulated within the population; however, when mutations are deleterious the population which is able to eliminate or maintain the mutation at low frequencies will best survive. Although a wide range of selection coefficients were examined, few mutations in a population that are not neutral are advantageous (Eyre-Walker & Keightley, 2007). The majority of mutations are lost within a population due to random genetic drift, however an advantageous mutation which is strongly selected for is less likely to be lost (Eyre-Walker & Keightley, 2007). Because of the rarity of advantageous mutations, it is uncommon for a selection coefficient to be measured at 1.2 or larger in a natural population. These experiments were conducted with changes to the degree of dominance, number of generations, and population size.

6.2.3 Altering parameters – sex ratio:

In the second set of simulations, the sex ratio was changed incrementally between 0.1 – 0.9, while selection was kept constant with either beneficial (greater than one), or deleterious (less than one) mutations. When mutations are strongly deleterious (ie selection parameter set to 0.8), mutations will quickly be lost from the population over a small number of generations (100). Therefore, the selection parameter was increased to midly deleterious mutations, while changing population sizes and number of generations to establish the optimal conditions to show accumulation of deleterious mutations across different mating systems.

6.3 Results and Discussion

6.3.1 Varying selection with five mating systems over time:

In the first set of simulations, five different mating systems were examined to determine how each population reacted to both beneficial and slightly deleterious mutations. Summary given in Table 6.3.

Table 6.3. Five different mating systems, description of their genetics, and the abbreviated name they will be referred to for the simulations. Diploids can also be referred to in literature as diplodiploids, as both males and females are diploid; therefore the abbreviated name in the simulations follows this longer name for consistency with other model names.

Mating System	**Description**	**Model Name**
Haplodiploid	Diploid females produced by fertilization; haploid males developed from unfertilized eggs	HD
Diploid (diplodiploid)	Diploid females and males developed from fertilized eggs	DD
Haplonty	Haploid individuals with a brief diploid recombination phase	H-R
Clonal haploids	All individuals are considered haploid females, with offspring identical to parent	H-C
Clonal diploids	All individuals are considered diploid females, with offspring identical to parent	H-D

Three replicates were taken for each mating system, for 100 generations in a population size of 100 individuals. Selection was modelled for completely dominant, slightly deleterious (0.95), and strongly beneficial (1.2) mutations, at a 1:1 sex ratio (0.5). It should be noted that in the clonal haploid mating system model, there is neither a sex ratio nor a dominance value. In addition, although there is a dominance level in the clonal diploid mating system model, all individuals in the population are considered to be female, eliminating the possibility of a sex ratio. The average divergence is depicted on the y-axis of all graphs, representing how quickly the mutation spreads through the population. When the mutation is beneficial, high divergence is advantageous, whereas if the mutation is deleterious, low divergence is advantageous. Results are provided in Figure 6.5.

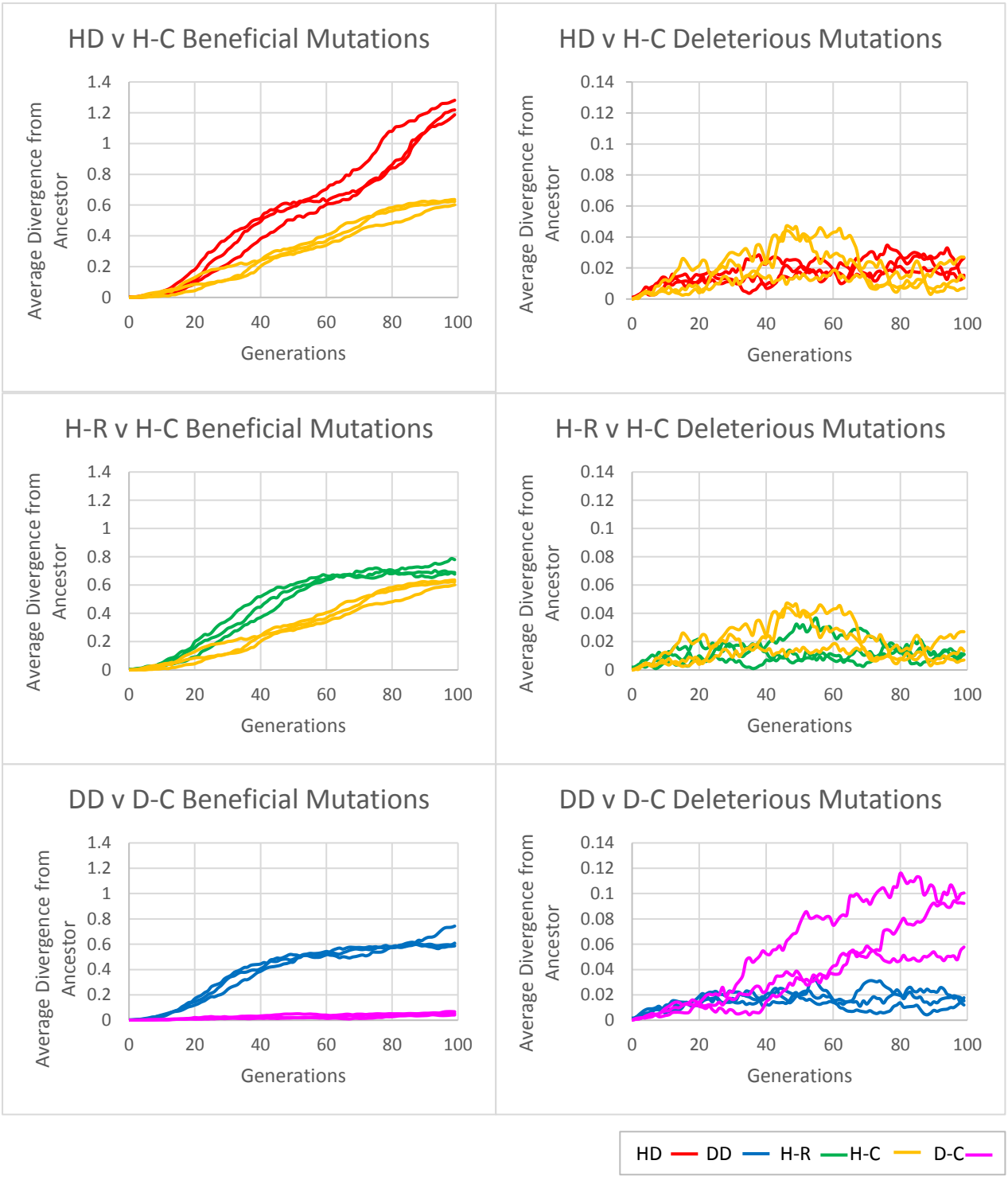


Figure 6.5. The average divergence from ancestor is depicted for five mating systems (HD, DD, H-R, H-C, and D-C), in a population of 100 individuals for 100 generations. There was a 1:1 sex ratio, and dominant mutations. Selection is measured with coefficients less than one (0.95) indicating deleterious mutations, and greater than one (1.2) representing beneficial mutations. A higher average divergence is best when mutations are beneficial, and a lower average divergence is best when mutations are deleterious. As a result, HD populations performed best.

As illustrated by Figure 6.5, dominant strongly beneficial mutations are rapidly accumulated in haplodiploids when compared to the other four mating systems. This is represented by a higher divergence from ancestor value (approximately 1.3, compared to the next highest value of approximately 0.8). As the mutations are beneficial, the individual wants to acquire more mutations, so this is positive for the haplodiploid mating system. In comparison, dominant deleterious mutations were maintained at a low equilibrium in haplodiploid, diploid, haplonty, and clonal haploids. However, clonal diploids accumulated deleterious mutations (approximately 0.1, compared to the lower values of approximately 0.02). Clonal diploid populations are influenced by the Meselson effect, where two alleles in an asexual diploid individual evolve over time to become different from one another (Welch & Meselson, 2000). This indicates how necessary a recombination phase is for the divergence and fixation of mutations in a population. The inability to add recombination into the clonal systems means beneficial mutations do not reach a high level of fixation, and deleterious mutations quickly spread through the population. This again provides support for the haplodiploidy mating system providing a fitness advantage, due to low accumulation of deleterious mutations.

6.3.2 Varying selection:

In the second set of selection simulations, five replicates were taken for the five aforementioned mating systems (haplodiploid, diploid, haplony, clonal haploids, and clonal diploids) over 100 generations in a population size of 100 individuals. Selection was modelled at 0.05 increments between strongly deleterious (0.8) and strongly beneficial (1.2) mutations. These mutations were fully dominant, and a 1:1 sex ratio (0.5) was applied (Figure 6.6). As before, the average divergence is depicted on the y-axis of all graphs, representing how quickly the mutation spreads through the population.

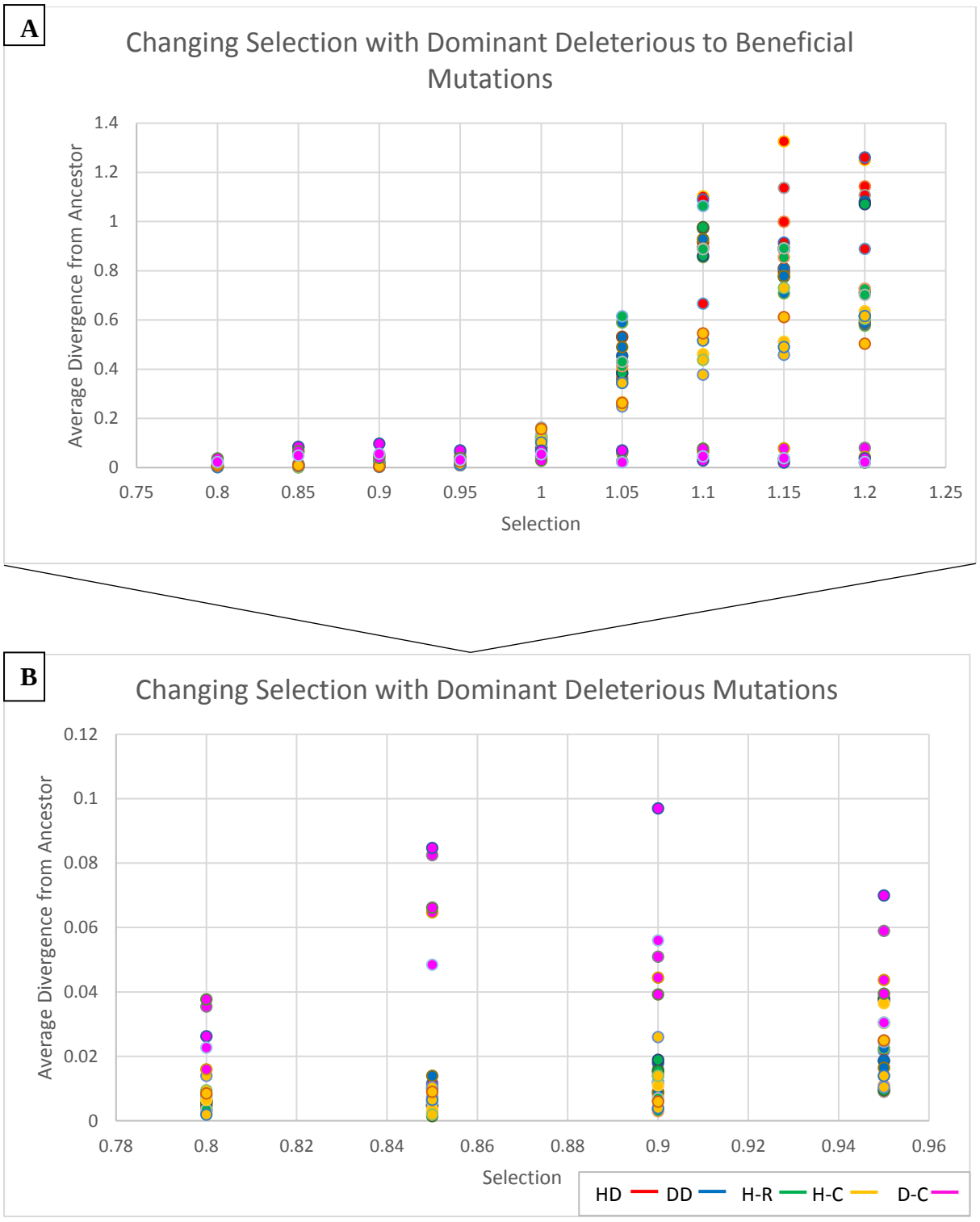


Figure 6.6. The average divergence from ancestor is depicted for five mating systems (HD, DD, H-R, H-C, and D-C), in a population of 100 individuals for 100 generations. There was a 1:1 sex ratio, and dominant mutations. Selection varied between deleterious (0.8) to beneficial (1.2) (Figure 6.6a). A higher average divergence is best when mutations are beneficial, and a lower average divergence is best when mutations are deleterious. Selection was "zoomed-in" between 0.8 and 0.95 to highlight the area where mutations are approaching neutrality (Figure 6.6b). This area was difficult to distinguish between the mating systems in the original figure. Once again, HD populations performed best.

When mutations were dominant and beneficial (greater than one), mutations are rapidly accumulated in haplodiploids (Figure 6.6a). This is represented by a higher divergence from ancestor value, compared to some of the other mating systems. When the mutation is beneficial, high divergence is advantageous, whereas if the mutation is deleterious, low divergence is advantageous. Therefore the acquisition of beneficial mutations is positive for the haplodiploid mating system.

When mutations were dominant and deleterious (less than one), mutations are accumulated in clonal diploids, but not the other mating systems (Figure 6.6b). As these mutations are deleterious, it is not in the individual's best interest to accumulate these mutations, depicted by a higher average divergence from ancestor. In comparison, dominant deleterious mutations were maintained at a low equilibrium in haplodiploid, diploid, haplonty, and clonal haploids. In the theory of nearly neutral molecular evolution, there is the assumption that some new mutations are selectively neutral and remain in the population, while the rest of mutations are deleterious and will be eliminated (Ohta, 1992). Therefore, mutations around zero are considered borderline mutations, and this is illustrated in the "zoomed-in" region of slightly deleterious alleles. Under this theory at the molecular level, random fixation of very slightly deleterious alleles contributes to a large portion of mutant substitutions (Ohta, 1992).

Table 6.4 was created to compare evolutionary consequences in the sexual mating systems (haplodiploidy, diploidy, and haplonty), considering variation in sex ratio, population size, and the selective effect and dominance relationship of new mutant alleles. This table illustrates the predicted effects of average divergence, as well as the observed effect for each changing parameter.

Table 6.4. Expected and observed outcomes on average divergence from ancestor in haploidiploid (HD), diploid (DD), and haploid with recombination (H-R) mating systems. Refer back to Table 6.2 for further descriptions of haploid, diploid, and haplodiploid mating systems with the addition of beneficial and deleterious mutations. Refer to Figures 6.5 – 6.8 for a graph of the observed effect on average divergence, however, not all results are included as figures.

Parameter	**Expected Effect on Average Divergence**	**Observed Effect on Average Divergence**
Population size increased	Would increase with beneficial mutations and decrease with deleterious mutations	As expected
Changes in sex ratio; beneficial mutations; dominant	Highest in H-R, followed by HD, and lowest divergence in DD	Highest in HD, much lower for H-R and DD (which had the lowest divergence)
	At high female sex ratio (0.1), effect on HD would be less compared to high male sex ratio (0.9)	Sex ratio was higher in HD when more equivalent, and lower in skewed sex ratios
Changes in sex ratio; strongly deleterious mutations; dominant	Highest in DD, lowest in H-R	Not clearly differentiated
Changes in sex ratio; beneficial mutations; co-dominant	Divergence would be half as high compared to beneficial mutations that were dominant	As expected
	Pattern same as beneficial dominant – Highest in H-R, lowest in DD	Not clearly differentiated, but appears to follow the expected pattern
Changes in sex ratio; strongly deleterious mutations; co-dominant	Divergence would be twice as high compared to deleterious mutations that were dominant	As expected
	Pattern same as deleterious dominant – Highest in DD, lowest in H-R	As expected
Changes in sex ratio; beneficial mutations; recessive	Highest divergence in H-R, lowest divergence in DD	H-R and HD display equally high divergence, with DD divergence substantially lower
Changes in sex ratio; strongly deleterious mutations; recessive	Highest in DD, lowest in H-R	As expected

Changes in selection; dominant	At low selection coefficient (highly deleterious), mutations unable to fix in any population so very low divergence	As expected
	As the selection coefficient increases (becomes beneficial), H-R will have highest divergence, DD will have lowest divergence	Divergence highest in HD, lowest in DD; and there is an unexpected dip at selection coefficient 1.5
Changes in selection; co-dominant	At low selection coefficient (highly deleterious), mutations unable to fix in any population so very low divergence	As expected
	As the selection coefficient increases (becomes beneficial), H-R will have highest divergence, DD will have lowest divergence	Not clearly differentiated; however as in selection dominant there is an unexpected dip at selection coefficient 1.5
Changes in selection; recessive	At low selection coefficient (highly deleterious), mutations unable to fix in any population so very low divergence	As expected
	As the selection coefficient increases (becomes beneficial), H-R will have highest divergence, DD will have lowest divergence	DD has the lowest divergence, H-R and HD are relatively equivalent, with the exception of a dip at selection coefficient 1.5 in H-R

Simulations were performed for dominant, co-dominant and recessive mutations, with selection varying from very deleterious (0.3) to very beneficial (1.8). Five replicates were taken for haplodiploid, diploid, and haplonty mating systems. Sex ratio was 1:1, and population size was 10 000 individuals over 100 generations (results not shown). It was anticipated that very deleterious mutations would not accumulate in the population in any of these three mating systems, and instead be maintained at low equilibrium frequencies. This was true for haplodiploid, diploid and haplonty mating systems, with dominant, co-dominant and recessive deleterious mutations.

However, it was expected that when mutations are beneficial, they would accumulate and spread through the population. It was anticipated that haplonty individuals would diverge at the fastest rate with beneficial mutations, as there is only one copy of every allele. Therefore immediate expression will lead to an increased fitness of the individual and its offspring. Unexpectedly, the haplodiploid mating system performed better than haplonty when the mutations were dominant, and relatively equivalently when the mutations were co-dominant and recessive. This is likely due to the extra copies of genes in haplodiploid individuals compared to haplonty providing more opportunities for beneficial mutations. In comparison, diploid individuals had to lowest divergence when beneficial mutations. This is likely a consequence of having two copies of each allele meaning only half as many mutations are passed on to the offspring, when compared to the haplonty mating system.

Taken together with the results presented in Figure 6.5 and 6.6, haplodiploidy is best at accumulating beneficial mutations and eliminating deleterious mutations at all dominance levels when there is an equal 1:1 sex ratio. Therefore, this was further explored with varying sex ratios to establish if this holds true in other situations.

6.3.3 Varying sex ratio with beneficial mutations:

In the first set of sex ratio simulations, five replicates were taken for haplodiploid, diploid, and haplonty mating systems. This was performed for dominant, co-dominant and recessive mutations, with sex ratio varying between highly female biased (0.1) to highly male biased (0.9). Selection was beneficial (1.2), and population size was 10 000 individuals over 100 generations (Figure 6.7).

Many preliminary simulations were performed with varied selection coefficients, number of generations, and population sizes (data not shown). There was little difference with number of generations, therefore 100 generations was selected for consistency. In addition, a similar pattern was observed for smaller-sized populations as for those with 10 000 individuals, however average divergence from ancestor became larger with increasing population size. Mutations spread more quickly when there is a larger number of individuals in a population, therefore divergence will be increased.

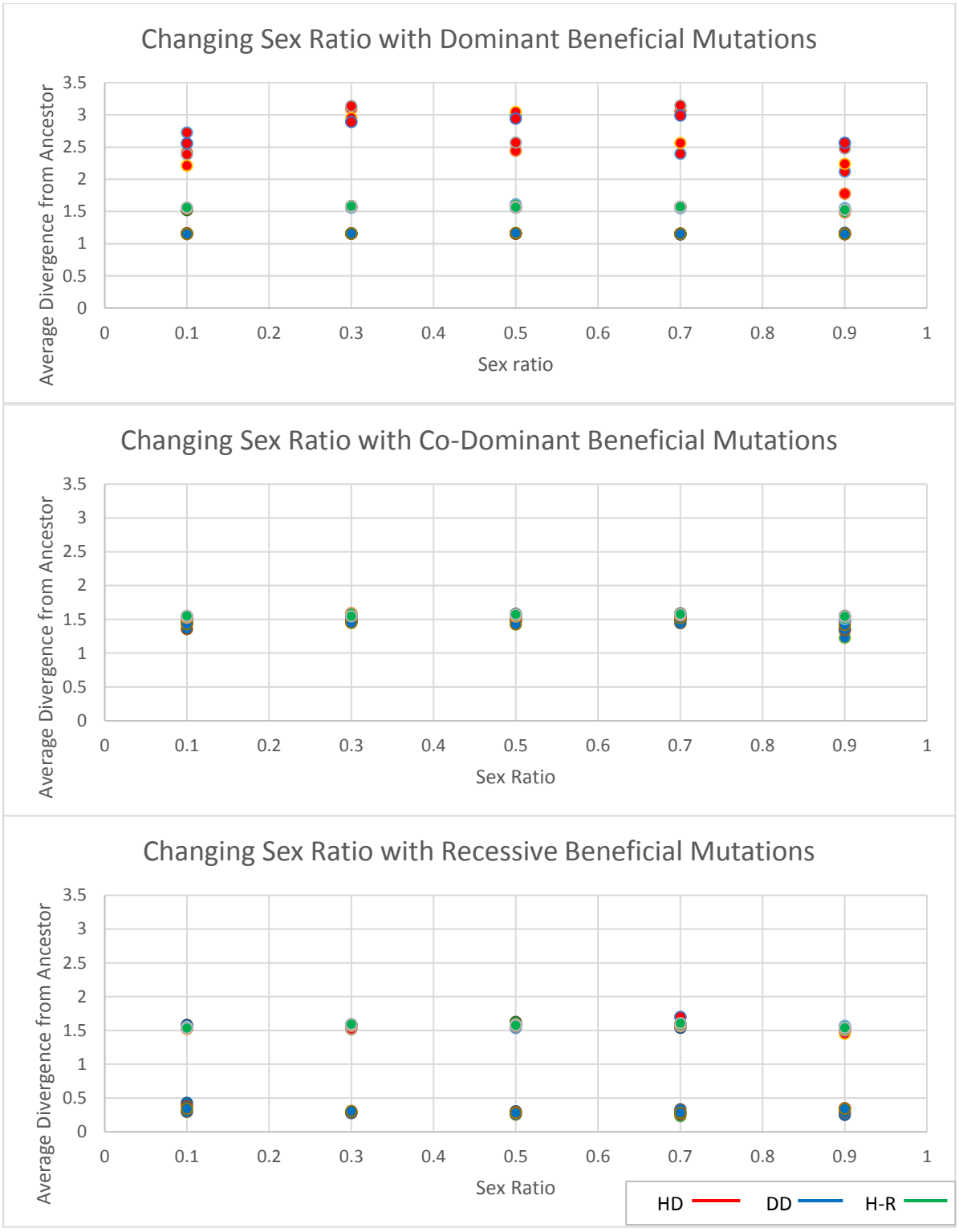


Figure 6.7. The average divergence from ancestor is depicted for three mating systems (HD, DD, and H-R), in a population of 10 000 individuals for 100 generations. Sex ratio varied between female biased (0.1) to male biased (0.9). New mutations were beneficial (1.2) and degree of dominance varied. Results were clearest with dominant mutations, and HD populations had the highest divergence indicating accumulation of beneficial mutations at all sex ratios.

As in Section 6.3.2, it was expected that haplonty populations would have the highest divergence from ancestor when exposed to recessive beneficial mutations. As previously described, there is only one copy of each allele in haploids; therefore a beneficial mutation would be immediately expressed and rapidly passed from parent to offspring. In comparison, in a haplodiploid population, the recessive beneficial mutation would only be immediately exposed in males but not females, and could be stochastically lost while still at very low frequency. The masking effects in a diploid population means only half as many mutations will be passed on to the offspring; therefore divergence from ancestor will be lower. I anticipated that sex ratios would have an effect on the level of divergence, with haplodiploid divergence higher when sex ratio was male-biased, and lower when female-biased. When mutations are recessive, the abundance of haploid males in the population will allow the beneficial mutations to be passed through the generations at a high frequency.

When mutations were beneficial and co-dominant, it was difficult to distinguish between the three mating systems (Figure 6.7). In both dominant and recessive simulations, the diploid mating system had the lowest divergence from ancestor, as expected. Results were clearest with dominant beneficial mutations, and showed that haplodiploids have the highest divergence at all sex ratios (between approximately 2.5 to 3, compared to haplonty at 1.5). This result was unexpected, as it was predicted haplonty would faster accumulate dominant beneficial mutations. This is explained by the presence of extra copies of the genes in the haplodiploid population compared to haptonty, which provides more opportunities for beneficial mutations. Average divergence was equivalent between haplodiploids and haplonty when exposed to beneficial recessive mutations. This indicates that haplodiploids are best at accumulating beneficial mutations compared to haplonty and diploids. Refer back to Table 6.4.

6.3.4 Varying sex ratio with deleterious mutations:

In the second set of sex ratio simulations, five replicates were taken for haplodiploid, diploid, and haplonty mating systems. This was performed for dominant, co-dominant and recessive mutations, with sex ratio varying between highly female biased (0.1) to highly male biased (0.9) in a population size of 10 000 individuals over 100 generations (Figure 6.8). As previously stated, preliminary simulations were performed with varied selection coefficients, number of generations, and population sizes (data not shown). It was established that for deleterious mutations, a selection coefficient of 0.95 best distinguished the level of divergence for the three mating systems. This mutation rate could be considered biologically applicable in wild populations.

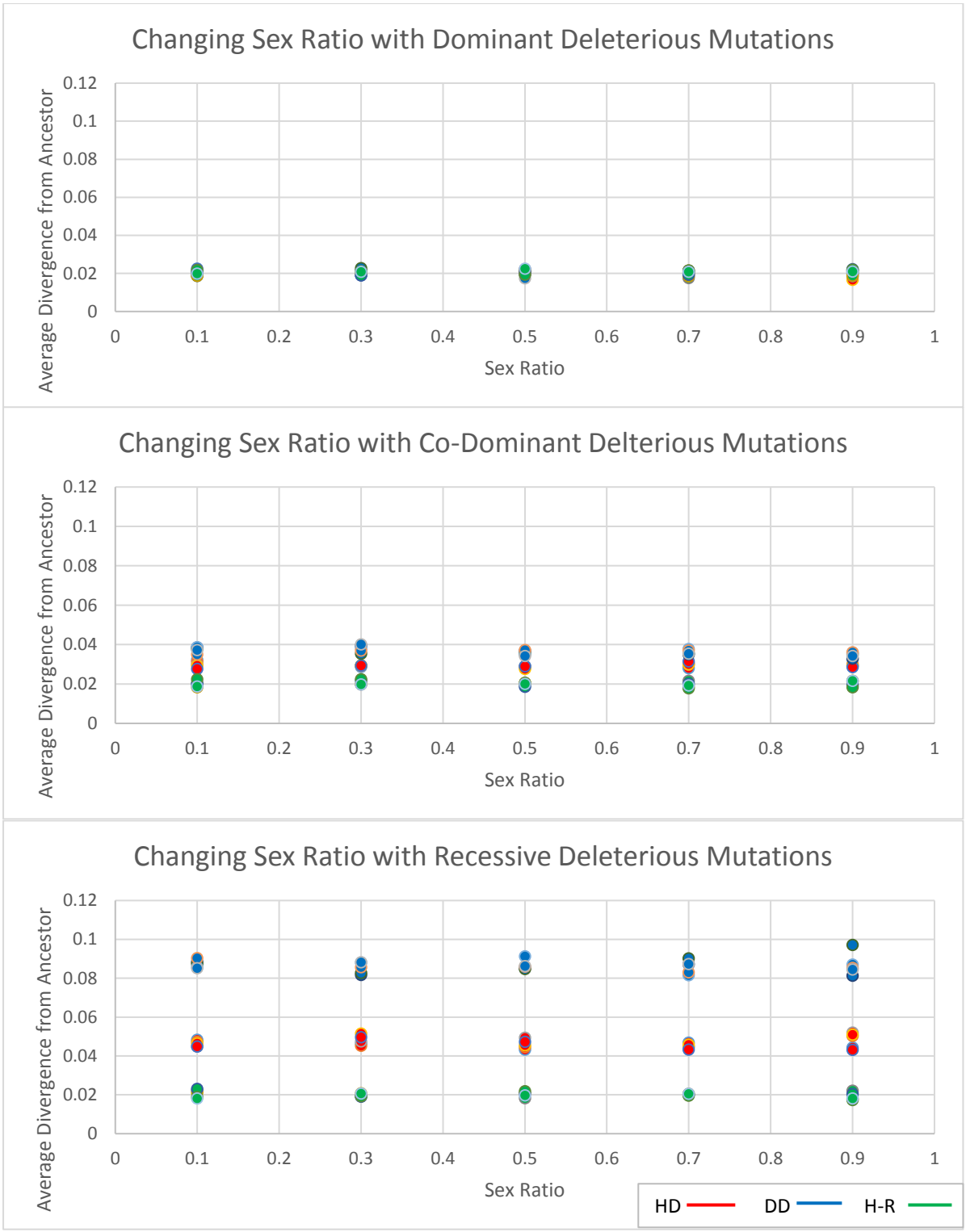


Figure 6.8. The average divergence from ancestor is depicted for three mating systems (HD, DD, and H-R), in a population of 10 000 individuals for 100 generations. Sex ratio varied between female biased (0.1) to male biased (0.9). New mutations were deleterious (0.95) and degree of dominance varied. Results were clearest with recessive mutations, and DD accumulated deleterious mutations, represented by high divergence. In comparison, HD had a low level of accumulation of deleterious mutations at all sex ratios, however this value was higher than in the H-R populations, which accumulated the fewest deleterious mutations.

As in Section 6.3.2, it was expected that in the presence of deleterious mutations, diploids would have the highest divergence from ancestor, and haplonty would have the lowest divergence. In a large population, deleterious mutations will be masked in diploids due to "duplicated" alleles, and these mutations will be passed to offspring and will persist in the population. However in haplodiploid and haplonty populations, deleterious mutations will be expressed and removed. Members of those populations that survive will have a higher fitness to pass onto their offspring. As such, the divergence will be less, especially in haplonty as removal will occur for both males and females, whereas in haplodiploids only males are haploid and benefit from expression.

The level of divergence was not clearly defined in dominant deleterious mutations. However, exposure to co-dominant and recessive deleterious mutations, illustrated higher divergence in diploid populations, as expected. Diploids are able to mask deleterious mutations, therefore the individual can survive and pass down the deleterious mutation to their offspring. The mutations are not purged at a high rate and therefore remain in the population at low frequencies. Consequently, the mutation persists for a longer period of time and becomes more diverged. Results were clearest with recessive deleterious mutations in Figure 6.8. Diploids accumulated deleterious mutations at a faster frequency (approximately 0.09), whereas haplodiploids and haplonty accumulated deleterious mutations at a lower rate (approximately 0.05 and 0.02, respectively), across all sex ratios. The haplonty population had the lowest divergence due to the immediate expression and removal of deleterious mutations in all individuals, whereas haplodiploids had an intermediate effect. This is due to deleterious mutations being exposed and removed in the males, but masked and transmitted through the populations by the females. Refer back to Table 6.4.

6.3.5 Differences between models:

To test the consistency of the above results, two models were designed with the same information, but differed slightly in the way fitness was simulated. Three replicates were taken for a population size of 100 individuals over 1000 generations, with an equal 1:1 sex ratio. Selection was modelled at slightly beneficial (1.05) and very slightly deleterious (0.999). Mutations were either fully dominant or fully recessive. The first model used the Perl program designed in this chapter and provided in Appendix 2, and the second model used the programming language C. Figure 6.9 compares haplodiploids and diploids under identical parameters with the following pattern in both: haplodiploids were better able to accumulate beneficial mutations when compared to diploids, and that the opposite was true regarding deleterious mutations. The average divergence from ancestor was marginally higher for the C program, which I expect is due to slight differences in how the program was coded.

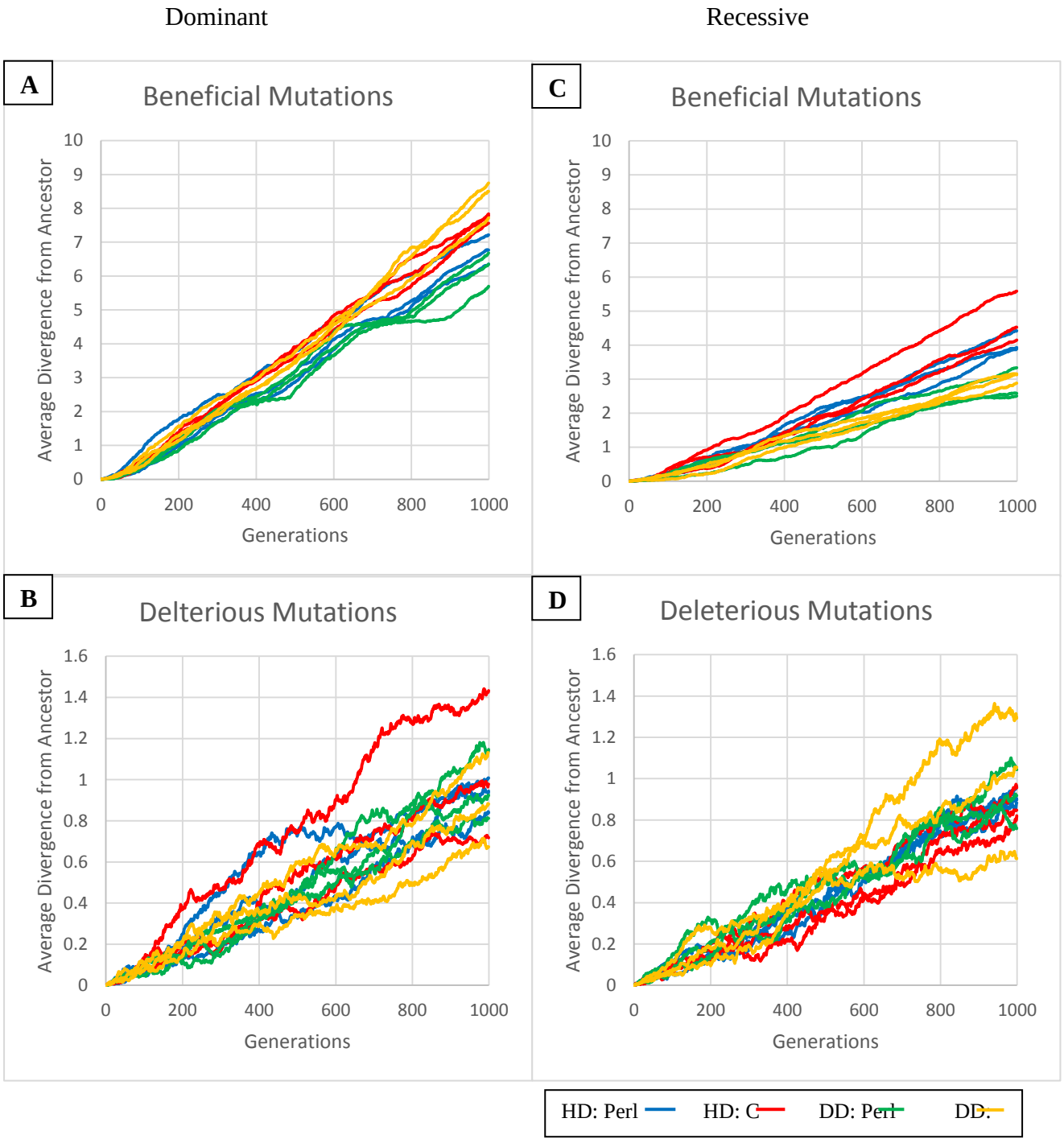


Figure 6.9. The average divergence from ancestor is depicted for two mating systems (HD and DD), in a population of 100 individuals for 1000 generations. There was a 1:1 sex ratio, and mutations were modelled as (a) dominant and slightly beneficial, (b) dominant and slightly deleterious, (c) recessive and slightly beneficial, and (d) recessive and slightly deleterious. Two programs, Perl and C, were compared. Under all conditions and both programs, haplodiploids were better able to accumulate beneficial mutations, and eliminate deleterious mutations compared to diploids.

6.4 Conclusions

This chapter explored the rates of accumulation of beneficial and deleterious mutations and simulated populations. Five mating systems (haploidploidy, diploidy, haplonty, clonal haploid and clonal diploid populations) were examined at varying selection coefficients – from strongly deleterious to strongly beneficial mutations. The y-axis in Figures 6.5-6.8 illustrate the average divergence from ancestor. When mutations are beneficial, a high divergence is advantageous, whereas when mutations are deleterious, a low divergence is optimal. For beneficial mutations, all populations accumulate mutations, however in a population size of 100 individuals over 100 generations and an equal sex ratio, haplodiploid individuals were most adept at accumulating beneficial mutations and keeping deleterious mutations at a low frequency within the population. In comparison, when mutations were deleterious, few mating systems are accumulate these mutations. Deleterious mutations are quickly exposed and removed from the population in haploid males, increasing fitness of the survivors and their offspring. In comparison, diploids mask recessive deleterious mutations due to having "duplicated" copies of alleles. As such, these deleterious mutations are kept in the population. Due to a lack of recombination, clonal populations were unable to accumulate beneficial mutations but had a higher frequency of deleterious mutations.

Varying sex ratios from strongly female-biased to strongly male-biased were examined with deleterious and beneficial mutations at differing levels of dominance across three mating systems (haplodiploid, diploid, and haplonty). When mutations were beneficial, haplodiploids had a higher divergence from ancestor, indicating the accumulation of mutations. However, when mutations were deleterious, diploids accumulated these mutations, which would have a negative impact on their survival. In comparison deleterious mutations remained at a low frequency in haplodiploids, and an even lower frequency in haplonty populations. Haplodiploid

males are able to expose and remove the deleterious mutations, whereas both males and females can expose and remove deleterious mutations in haplonty populations.

In the next chapter, these results will be expanded to include haplodiploid and diploid mating systems within one population. The population will begin as diploid, however the introduction of a cytoplasmic maternally-transmitted element into the population will turn males who inherit this element from their mothers, haploid. This will be performed to have a direct competition between these two mating systems to assess whether haplodiploidy shows more adaptive evolution compared to diploidy. Based on the results from this chapter, it is expected that the element will spread through the population and after a chosen number of generations, the majority of the population will have changed from diploidy to haplodiploidy.

Chapter 7: Haplodiploid and Diploid Direct Competition

7.1 Introduction

Previous results (see Chapter 6) illustrated the advantages of haplodiploidy using Perl simulations due to the immediate exposure and removal of deleterious mutations as well as faster accumulation of adaptive mutations compared to other genetic systems. However, although haplodiploidy may be advantageous for the individual to evolve to this mating system for survival, it may not readily spread through the population. In fact, the strategy most likely to evolve is the one with the most evolutionary stable strategy (ESS). In this chapter I will explain the concept of an ESS, followed by the evolution of haplodiploidy. This will further discuss previous notions from Chapter 6 regarding the advantages and disadvantages of haploidiploidy, as well as outlining mechanisms which can turn an individual haplodiploid. Finally, a model will be used to directly compete diploids and haplodiploids in the same program to determine whether haplodiploidy will spread through a diploid population.

7.1.1 Evolutionary stable strategy:

a. Concept:

John Maynard Smith (1974) expanded upon previous ideas and research (Maynard Smith & Price, 1973, Geist, 1966, Hamilton, 1967, MacArthur, 1965) to propose the concept of an evolutionary stable strategy (ESS). This work was first carried out with regards to animal conflicts, however the underlying ideas have been applied to many other evolutionary phenomena, such as the Prisoner's dilemma (Lindgren, 1992), the social sciences (Axelrod, 1984), and game theory (Maynard Smith, 1992b). This concept is solely dependent on individual advantage and is not impacted by interactions with nature. When the best strategy

for an individual is reliant on the behaviour of others, ultimately the strategy that they adopt will be an ESS (Maynard Smith, 1992a).

b. Evolutionary invasion analysis:

One of the main issues when modelling genetic systems is the level of genetics explicitly included within that model. Optimality arguments are used in an attempt to predict evolution, on the basis that a particular trait will be maximized over time (Otto & Day, 2007). These are a measure of fitness, normally assessed though either population growth rate or the basic reproduction number (Mylius & Diekmann, 1995). In a steady state, both these values can be considered maximal. One refers to the environment, determined by the resident; whereas the other refers to the type of invader in a population (and the maximum is determined with respect to this value). Fitness measure is reliant on the way density dependence acts, and when examining life-history traits, population growth is limited by this density dependence (Mylius & Diekmann, 1995). However, even if something is beneficial for the individual, it does not necessarily mean that it will evolve in the population.

Many hypotheses, including some game-theoretic models are based on principles of evolutionary invasion analysis, but do not overtly outline the genetic model upon which they are based (Otto & Day, 2007). Genetic and ecological details such as number of individuals or genes can be ignored in place of observing social interactions of the requisite species. One type of evolutionary invasion analysis, adaptive dynamics, attempts to elucidate evolutionary forces with a focus on explicit mathematical framework and mutational steps. Evolution is considered a mutation-limited process, where there is an equilibrium in the population when only a single allele remains. Introduction of a new allele through mutation either replaces the current allele,

with a new equilibrium reached, or is lost. This process continually repeats itself, and adaptive dynamics provides a clear stochastic model of this process (Otto & Day, 2007).

c. Example – hawk/dove:

Conflict situations result in strategies where an individual can cause serious injuries to another, or strategies where injury is impossible and the winner is the individual who is able to fight the longest (Maynard Smith & Price, 1973). A common example of an ESS-based evolutionary model is given in Table 7.1, where it was asked: what selection strategy is best for the individual? It should be noted that this is a simplified model, and the "best" outcomes, in terms of optimal average outcome for the population as a whole, is not necessarily best for an individual and therefore may not be one that evolves (Maynard Smith & Price, 1973).

Table 7.1. Average pay-offs using computer simulations of five strategies (Maynard Smith & Price, 1973). Green illustrates ESS, yellow is almost an ESS, and red is not an ESS. Only "Retaliator" is an ESS, with "Prober-Retaliator" almost an ESS. In the other strategies, the pay-off for the opponent is not highest for the corresponding contestant. Evolution will occur to comprise mainly these two strategies, with the other three kept at low frequencies in the population by mutation.

		Opponent				
		Mouse	Hawk	Bully	Retaliator	Prober-Retaliator
Contestant receiving the pay-off	Mouse	29	19.5	19.5	29	17.2
	Hawk	80	-19.5	74.6	-18.1	-18.9
	Bully	80	4.9	41.5	11.9	11.2
	Retaliator	29	-22.3	57.1	29	23.1
	Prober-Retaliator	56.7	-20.1	59.4	26.9	21.9

Under natural selection, if most members of a population adopt the ESS, no invading "mutant" strategy would have a higher reproductive fitness (Maynard Smith & Price, 1973). A payoff may be a direct or indirect measure of fitness, and even if the fitness of a population may be maximised under certain conditions, the strategy will not be considered a competitive optimum

or ESS if a mutant strategy can invade (Parker & Maynard Smith, 1990). An ESS may be either a pure or mixed strategy; a mixed strategy is defined as adopting a "pure" strategy based on preassigned probabilities (Maynard Smith, 1974). Therefore a stable population may be genetically polymorphic (different frequencies of individuals adopting different pure strategies), or monomorphic (behaviour of individuals is appropriately random) (Maynard Smith, 1974). Overall, the ESS is to adopt a strategy that does not cause serious injury, but to escalate to serious injury if your opponent does (Maynard Smith & Price, 1973). However, there are some instances where an optimal ESS is not generated (Parker & Maynard Smith, 1990).

d. Example – sex ratio:

An important application of ESS thinking is the evolution of the sex ratio (Maynard Smith, 1992a). In most species there are an equal number of males and females, and equal sex ratios tend to evolve. One option seeks to explain this by evolving the sex ratio to maximize the number of encounters between individuals of the opposite sex. This is a "group selection" argument, and was proposed as an adaptive explanation for the prevalence of 1:1 sex ratios as optimal (Maynard Smith, 1992a). However, equal sex ratios are not best from an "optimise the population" perspective (ie fig wasps) (Cook et al., 2017). If group selection was strong and groups tended to "win" an ESS, then few males would be needed to successfully sustain the population. There would be an unequal sex ratio, therefore, optimisation at the population level is not necessary.

The second option taken from Fisher (and the one Maynard Smith deems correct) assumes genes can influence the sex ratio by favouring the number of offspring produced by one individual carrying the genes (Maynard Smith, 1992a). In a female biased population,

production of sons will lead to more grandchildren, with the opposite being true of daughters in a male-biased population. If there are equal numbers of males and females, offspring of both sexes are equally important making a 1:1 sex ratio the "evolutionary stable strategy". There may also be different costs associated with the production of sons or daughters (Maynard Smith, 1992a). Sex ratio theory based on ESS and Fisherian assumptions has been rigorously tested, and can generally explain sex ratios in nature very well (Frank, 1990).

7.1.2 Evolution of haplodiploidy:

There are many different mechanisms through which haplodiploidy may have evolved (Table 7.2). See (Blackmon et al., 2015) for a current theory of how haplodiploidy evolved due to genetic conflicts, as well as authors who have considered different theories for haplodiploidy evolution. Two of the most common methods to generate haplodiploids are arrhenotoky (unfertilized eggs develop into males), and pseudo-arrhenotoky (males develop from fertilized eggs but become haploid after paternal genome inactivation or elimination) (Sabelis & Nagelkerke, 1988). Although in both systems males are effectively haploid and only the maternal genome is passed down to their offspring (Sabelis & Nagelkerke, 1988), these two processes differ as arrhenotokous males are completely haploid throughout development, whereas pseudo-arrhenotokous males begin development as diploid, but lose their paternal genome and their genes are not passed down to offspring (Normark, 2004).

Table 7.2. Four hypotheses for the evolution and maintenance of haplodiploidy (Normark, 2004). Sex ratio control illuminates why haplodiploidy may occur, and the other three hypotheses describe the mechanisms behind how the process of haplodiploidy may occur.

Hypothesis	**Description**	**References**
Sex ratio control	There is maternal control over sex-ratio in haplodiploidy which leads to female-biased sex ratios	(Hamilton, 1967, Borgia, 1980)
	Haplodiploid individual has the advantage	
Maternal transmission advantage	Females have a transmission-genetic advantage if they can produce viable sons without the requirement of a male parent	(Brown, 1964, Hartl & Brown, 1970, Smith, 2000, Bull, 1979)
	Most successful mechanism	
	Haplodiploid individual has the advantage	
Deleterious mutation clearance	Selection during the haploid phase is most effective at removing deleterious mutations	(Smith, 2000, Goldstein, 1994)
	Haplodiploid population has the advantage	
Feminizing endosymbionts	Endosymbionts are transmitted maternally and eliminate the paternal genome; only the maternal genes are passed to offspring, resulting in a female-biased sex ratio	Primary reference not found, but see (Normark, 2004)
	Endosymbiont has the advantage but not the haplodiploid host	

Previous models of mating system evolution often compare different mating systems, in particular haploidy with diploidy, to identify the duration an individual remains in each phase due to the advantages of being either haploid or diploid for a prolonged period of time (Jenkins & Kirkpatrick, 1995). Theoretical studies which examined effects of deleterious mutations displayed different outcomes (Jenkins & Kirkpatrick, 1995). In a population which includes genome-wide mutations and selection, if there is no interbreeding between haploids and diploids, then diploidy is favoured when epistasis is strong between loci (Kondrashov & Crow, 1991). However, diploidy can be evolved without epistasis, and diploid systems can be favoured over haploid systems under "reasonable assumptions" of dominance and recombination (Perrot et al., 1991, Kondrashov & Crow, 1991, Bengtsson, 1992, Otto & Goldstein, 1992). The development of a two-locus model provided a basis for interbreeding between haploid and diploid individuals (Perrot et al., 1991). Diploid invasion was dependent on how deleterious the mutation was, and diploids only invaded when dominance degree of

each deleterious mutation was less than ½ (Perrot et al., 1991). A companion study (Jenkins & Kirkpatrick, 1994) referenced in Jenkins & Kirkpatrick (1995), developed a single locus model where lengths of haploid and diploid phases could be varied. It was determined that haploidy evolves when there is complete linkage, mutations are strongly deleterious, or mutations are partially dominant (Jenkins & Kirkpatrick, 1994).

Furthermore, evolution of life cycles were examined in a model that included mutation and selection in an interbreeding population (Jenkins & Kirkpatrick, 1995). The length of time spent in haploid and diploid phases can evolve, with a single genetic locus controlling the amount of time spent in each phase. Selection acts on deleterious mutations and results in the evolving life cycle. The number of deleterious mutations controls mortality rate of the individual. There are epistatic interactions between mutations and fitness is multiplicative across loci. Results of this genome-wide analysis again showed that the diploid phase in increased under biologically reasonable assumptions, regardless of the presence or absence of epistasis. In comparison, the haploid phase is favoured when mutations are highly deleterious or partially dominant. This supports the evolution of life cycles in the above models which examined interbreeding populations and selection on mutations at single-loci (Jenkins & Kirkpatrick, 1995). Therefore, to expand upon these concepts and examine mating systems from a different angle, I created a simulation model where two strategies (diploid and haplodiploid) could be competed directly (Section 7.2). The following sections discuss advantages of male haploidy that can be incorporated into these models, as well as difficulties of male haploidy, something which is not normally included in models.

7.1.3 Advantages for haplodiploid males:

a. Maternal transmission advantage:

In haplodiploid males, only the maternal genome is transmitted; therefore the maternal genes will occur twice as often in the gametes of haploid sons when compared to the gametes of diploid sons (Bull, 1979). This two-fold representation of maternal genes confers a selective advantage on females for the selection of haploid males when compared to biparental diploid sons. This provides higher identity-by-descent between a grandmother and her grandchild, an advantage which may compensate for the potentially lower fitness of the haploid male offspring. Once haploid males are established, selection must be towards mutations in maternal genes that produce haploid sons and against paternal genes attempting to restore diploid sons (Bull, 1979).

b. Maternal sex ratio control:

As previously discussed, there is an increased ability for haplodiploid individuals to control sex ratio (Sabelis & Nagelkerke, 1988). In mites, the Phytoseiidae are pseudo-arrhenotokous, where haploid males arise from fertilized eggs. However, the paternal genome is eliminated by an unknown underlying maternal control mechanism that distinguishes between the paternal and maternal genomes, prior to inactivation and elimination of the paternal genome (Sabelis & Nagelkerke, 1988).

c. Female reproductive assurance:

A final advantage of male haploidy is reproductive assurance for females in instances where a sexually compatible partner cannot be easily obtained (Gates & Nason, 2012). In figs (genus *Ficus*, family Moraceae), there is a mutualistically beneficial relationship with species-specific

pollinating fig wasps. Although *Ficus* is very diverse with greater than 750 species, individual species are found at low population densities. Consequently, dispersal between the fig and wasps is quite difficult. This can further be exemplified in many plants, as a requirement for survival is some sexual reproduction via outcrossing through the movement of pollen (often by an animal from one plant to another). Many of these plants, however, are self-compatible and can undergo selfing as well as outcrossing. Selfing increases gene transmission and provides reproductive assurance by eliminating the need of a "mate" for sexual reproduction (Gates & Nason, 2012). This is a similar process in haplodiploidy: since females can produce males from unfertilized eggs, if there is a lack of mates there is a shift towards reproduction in this manner so that the population is still able to survive and thrive.

7.1.4 Difficulties of transitioning to male haploidy:

a. Exposure of deleterious alleles:

Ultimately the evolution of the haplodiploidy mating system is dependent on overcoming issues with development of haploid males (Borgia, 1980). The process of haploid male spermatogenesis is highly restrictive, which has likely limited the evolution towards haplodiploidy (Whiting, 1945, Hartl & Brown, 1970). It is necessary for diploids to have a low level of deleterious mutations, otherwise there would be issues with haploid survival (Whiting, 1945, Hartl & Brown, 1970). Inbreeding assists haplodiploidy through purging deleterious mutations which results in a reduced mutational load, as well as reducing the effectiveness of recombination (Smith, 2000). Diploid individuals carry recessive lethal mutations, and if these genes are passed to an unfertilized haploid egg, there is incomplete parthenogenesis and mortality (Whiting, 1945, Hartl & Brown, 1970).

b. Parthenogenesis and dosage compensation:

In a normal diploid population, genes favouring parthenogenesis will spread through the population (Whiting, 1945, Hartl & Brown, 1970). However, in most diploid species there are mechanisms like dosage compensation which act as a barrier to parthenogenesis. Parthenogenesis results in a reduced number of chromosomes in haploid males (Whiting, 1945, Hartl & Brown, 1970). Due to the imbalance between two large gene rich X chromosomes in females, and only one X chromosome and one small, gene poor Y chromosome in males, individuals have evolved processes to equalise gene expression (dosage compensation) (Brockdorff & Turner, 2015, Kaiser & Bachtrog, 2010). For a haplodiploid male to develop, dosage compensation mechanisms must be overcome (Whiting, 1945, Hartl & Brown, 1970). Haplodiploidy may take away some of these barriers, as females are able to make males without a father. Genes favouring parthenogenesis are likely to be transmitted in genetic systems where the initial viability of many eggs is poor (Normark, 2006), and may particularly occur in haplodiploids due to the two-fold increase in relatedness of mothers to their haploid offspring (Brown, 1964). However, it should be noted that full parthenogenesis is considered an evolutionary dead-end (Maynard Smith, 1978), as referenced in (Lynch & Gabriel, 1983). Due to the two-fold cost of sex in haplodiploidy, population size would be increased fastest by cloning (Maynard Smith, 1992a). However, males are still being made at a higher frequency in haplodiploidy, something which would not occur so readily in cloning. As such, adaptive evolution will favour haplodiploidy over asexual mating systems (Maynard Smith, 1992a).

7.1.5 Paternal genome elimination as an intermediate step:

a. Definition:

Haplodiploidy most often occurs through arrhenotokous reproduction, where unfertilized eggs develop into haploid males and fertilized eggs develop into diploid females (Normark, 2004).

However, a haplodiploid mating system can also be created through paternal genome elimination (PGE; pseudo-arrhenotoky) (Normark, 2004). PGE is frequent in arthropods, and often occurs by infection with endosymbionts (ie *Wolbachia*) (Cordaux et al., 2011). In groups of arthropods, PGE occurs less often than haplodiploidy (Yokogawa & Yahara, 2009). As such it was suggested that PGE acts as a transitionary step towards haplodiploidy, a theory which has not yet been explicitly tested (Schrader & Hughes-Schrader, 1931), or that that PGE may evolve into arrhenotoky which would eliminate the need for males and any paternal contribution (Normark, 2004).

b. Endosymbionts in arthropods:

Endosymbionts are a factor which have been known to manipulate host reproduction affecting mating systems in arthropods (Cordaux et al., 2011). For example, the common endosymbiont *Wolbachia* infects arthropods and causes PGE in the infested individual (Cordaux et al., 2011). Although nuclear volumes are consistent between males and females in organisms, there is a large size discrepancy in cytoplasmic volume (Normark & Ross, 2014). Due to the egg having a much greater cytoplasmic volume compared to sperm, often including elements with their own genome, cytoplasmic genomes are transmitted via females. As a result, unlike in the nucleus, there is no direct fitness on the cytoplasmic elements in males. Consequently, male fitness is often sacrificed for female fitness in one of four ways: cytoplasmic incompatibility, male-killing, feminization of genetic males, and parthenogenesis induction (Normark & Ross, 2014) (Table 7.3).

Table 7.3. *Wolbachia* is the only known endosymbiont to induce all four types of reproductive manipulation leading to haploid males following initial development as diploids (Cordaux et al., 2011). Each type of reproductive manipulation leading to male haploidy is described in detail.

Reproductive Manipulation	**Process**	**Description**
Cytoplasmic incompatibility	Favours infected female reproduction	Causes sterility due to sperm and egg incompatibility during mating between an infected and uninfected individual
		Maternally inherited endosymbionts are transmitted
Male-killing	Distorts sex ratio	Male offspring death leads to highly female-biased sex ratio
		Differs from feminization and PI as death occurs in the (male) sex that does not vertically transmit endosymbionts
Feminization	Distorts sex ratio	Converts genetic males into phenotypic functional females, which leads to a highly female-biased sex ratio
		Mechanism largely unknown, however possible that interactions between endosymbionts with different molecular pathways create populations in which all *Wolbachia* infected individuals develop a female phenotype, and non-carriers develop as males
Parthenogenesis induction	Distorts sex ratio	Converts non-transmitting (genetic) haploid males into transmitting (genetic) diploid females, through chromosomal doubling in unfertilized eggs
		Infected females can then produce endosymbiont-transmitting female offspring without the use of males during sexual reproduction, promoting asexual daughter development (highly female-biased sex ratio)

c. Hypothesis one for paternal genome elimination evolution:

There is a high risk for male haploids to revert to diploidy if there are mutations transmitted through sperm which prevent PGE (Bull, 1979). As such, inheritance of genes controlling PGE from the mother may lead to the evolution of male haploidy (Bull, 1979). An XX-XO sex determination system may be able to evolve into a maternally controlled sex ratio system, allowing for PGE evolution (Haig, 1993). This XX-XO sex determination system is found in nature in species such as nematodes, where sperm with an X chromosome produce daughters, and without an X chromosome produce sons (Haag, 2005). X-linked genes favour higher

proportion of X-bearing sperm in males when compared to autosomal genes. If there is meiotic drive on the X chromosome in XO males, autosomal genes may evolve to exploit this system to be included in X-bearing sperm. This high level of X-bearing sperm leads to a highly biased female sex ratio, and an overall evolution to eliminate the paternal genome (haplodiploidy) (Haig, 1993).

d. Hypothesis two for paternal genome elimination evolution:

The second hypothesis involves the invasion of a maternally transmitted endosymbiont which turns diploid male zygotes into haploids (Haig, 1993) (Table 7.4). Normark's original model is based off Hamilton's (1993) observation that haplodiploid ancestors were commonly infected by symbionts. This hypothesis combines the maternal transmission advantage along with the association to maternal endosymbionts (Normark, 2004). Engelstädter and Hurst (2006) expanded upon Normark's model to examine the conditions which allow male haplodizers to invade the population. They found issues with the original model, namely it is never beneficial to be a male-killer when viability for haploid males is low, as the skewed female-biased sex ratio would reduce the fitness of infected females (Engelstädter & Hurst, 2006). Additionally, haploid male fertility must be high to generate conditions in which male-killers are beneficial for host females, which is unlikely in an initial population (Engelstädter & Hurst, 2006). Although Normark's model plus their expansions is plausible, evolution acting on both the bacteria and the host is necessary to drive haplodiploidy evolution by male-killers (Engelstädter & Hurst, 2006). Kuijper & Pen (2010) demonstrated that the assumptions made in the previous models would not lead to the persistence of haplodiploidy. Eventually the survival of haploidized males will select for maximal levels of haploid male viability and reallocate resources from infected hosts. This decreases the fitness benefits of infected hosts, as such, the endosymbiont is likely to be purged due to genetic drift (Kuijper & Pen, 2010).

Table 7.4. Models for the origin and adaptation of haplodiploidy providing further support for hypothesis two of the evolution of PGE through the invasion of a maternally transmitted endosymbiont turning diploid male zygotes haploid.

Model	**Mechanism**	**Outcome**
(Normark, 2004)	Cytoplasmic maternally transmitted element (male hapolidizer) that kills males by haploidizing them immediately after fertilization (reacts against the genome of incoming male-determining sperm (Ych))	Small number of haploid males survive, and the elimination of the paternal genome provides an advantage to the maternal host (increased transmission of her genome through haploid males)
		Provides an opportunity for the male-killing endosymbionts to become fixed in the population, which in turn may increase haploid male viability and result in a PGE genetic system
(Engelstädter & Hurst, 2006)	As before, but includes circumstances allowing maternally transmitted male-killing bacteria to invade and thrive in a population, alters the associated cost of the death of sons due to the higher female sex ratio, and the unequal gene transmission through offspring infected and uninfected females	As before, meaning a haplodiploid mating system may be favoured in the population
(Úbeda & Normark, 2006)	Analyze co-evolution between male haploidizers and its host by including variable transmission of male haplodizers and variable survivorship of haploidized males	Invasion of male haplodizers leads to selective pressures on bacterial transmission and host survivorship; regardless of whether gene control is cytoplasmic or nuclear, male haplodizers either become fixed or go extinct
		Selection on cytoplasmic genes increase their own transmission but decrease male host survivorship; selection on nuclear genes may increase or decrease bacterial transmission, but increase host survivorship
		PGE originates once selection acts to maintain male haplodizer transmission and host survivorship, keeping the population at this equilibrium
(Kuijper & Pen, 2010)	As before, with two proposed mechanisms through which male-killing endosymbionts can drive the evolution of haplodiploidy: spatially-structured populations which are highly inbreed, or a mutualistic relationship between endosymbionts and their hosts	It is nearly impossible for the haplodizing endosymbiont (and haplodizing males) to stably persist in a population longer than 20 000 to 30 000 generations when transmission is controlled by either the host or the endosymbiont

7.1.6 Competition model:

To further explore haplodiploidy and diploidy, I created a simulation model which allowed for direct competition between the two mating systems. I was interested in exploring that given the advantages incurred by haplodiploids (see Chapter 6), will this mating system be able to evolve from diploidy. The two programs were modelled as similarly as possible, with both males and females beginning development as a diploid. The only difference was the introduction of a maternally transmitted element causing haploidploidy in diploid males if they inherited the element to create competition between the two mating systems. Since haplodiploidy favours the mothers, as outlined in Section 7.1.3, the element was modelled as a maternally transmitted cytoplasmic element.

The aim of this model was to determine whether haplodiploidy is a better mating system to evolve, as it has proven to be an efficient system for removal of deleterious mutations and accumulating beneficial mutations. From this, the model could be examined through many generations to determine whether haplodiploidy is carried and converts all members of the population to this genetic mating system, or whether haplodiploidy is lost after a few generations and the population remains diploid.

7.2 Materials and Methods

7.2.1 Competition parameters:

As before, Perl 5 (Schwartz et al., 2017) was used to directly compare haplodiploidy and diploidy, within the same population. As in Chapter 6, number of genes was kept constant at 20, and mutation rate was kept constant at 0.001 throughout all simulations. There was a 1:1 sex ratio (0.5), in a population size of 100 individuals, and the simulation was run for 100 generations. Selection and dominance varied between simulations. These simulations differed from those presented in Chapter 6 in that some females initially carried a maternally transmitted element inducing haplodiploidy. The complete program is provided in Appendix 2, Figure 7.5.

7.2.2 Maternally transmitted element:

As outlined in the introduction, there are a variety of potential mechanisms which can be used to convert diploid males into haploid males. For these simulations, it was decided that the most straightforward way to model the two mating system competition was for individuals to begin as diploid, and introduce a maternally-transmitted element which caused male haploidy. The element had an initial starting rate in the population which illustrated the number of individuals, both male and female, who carried the element. All females, regardless of whether they carried the element or not would be diploid and remain in this state throughout all matings and generations. Diploid females who did not carry the element would mate with diploid males and produce diploid offspring (male and female). However, diploid females who carried the element would mate with diploid males and produce haploid sons; this amounts to paternal genome elimination. Haploid males are able to mate, and transmit their entire genome to offspring. Note that the reproductive assurance advantage of haplodiploid therefore does not apply, as the females must still find a suitable fit male for mating. The prevalence of this

element, determining the frequency at which it was transmitted through the population could be measured over a set number of generations. See Figure 7.1 for an illustration of how the competition Perl program was designed.

The maternally transmitted element causing paternal genome elimination was introduced at a starting rate of either 0.1, 0.5, or 0.9. If the mutation was completely neutral, due to genetic drift it would be expected that the element would be fixed 10% of the time, 50% of the time, or fixed 90% of the time, respectively. Strong variations from these values would indicate whether haplodiploidy or diploidy was favoured in the population, as the rate of loss or gain of the element would not be due to random chance (drift).

7.2.3 Initial diversity:

Following the first set of simulations (see Section 7.3.1), an initial diversity parameter for standing genetic diversity was included in the simulations. This represented the percentage of alleles that were initially mutated within the population prior to mating, and illustrates another difference between the competition parameters and those from Chapter 6. Without an initial diversity, there are no recessive deleterious alleles in the population to begin with. Haplodiploidy will expose and remove these alleles over a period of time as they accumulate in the population, however there would be no immediate initial effect. By introducing an initial diversity, females may already have deleterious alleles to be passed on to her sons, which would be a disadvantage to these males. Therefore, the initial diversity was included in the simulations to better resemble a transition from an initial diploid state with some standing genetic diversity to haplodiploidy.

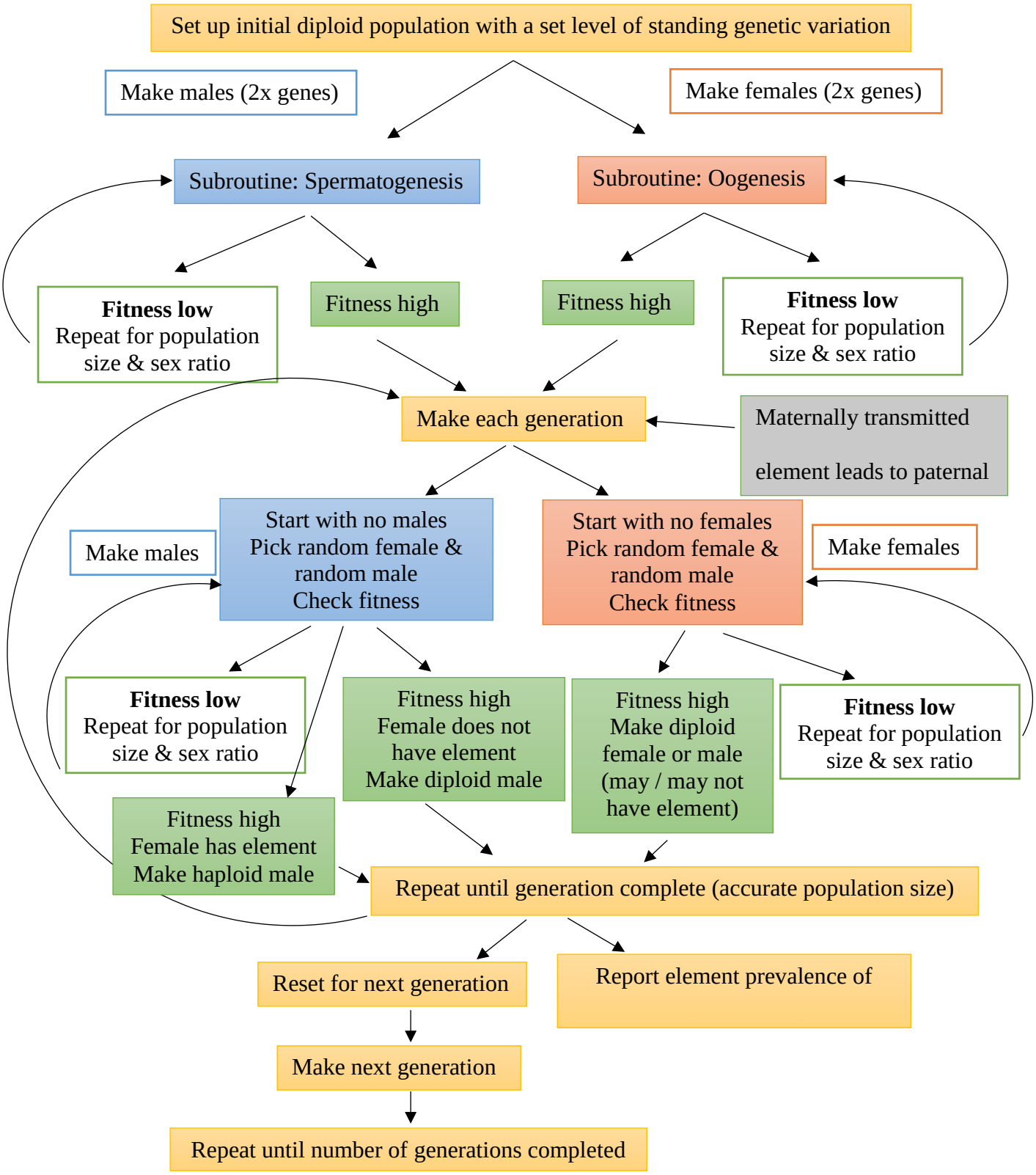


Figure 7.1. Design of Perl for a diploid population which turns some males haploid through the introduction of a maternally transmitted paternal genome elimination element. Rate of gain / loss of the element illustrates whether haplodiploidy or diploidy becomes the dominant genetic system. Compare with Figure 6.4 (haplodiploid mating system).

7.3 Results and Discussion

7.3.1 Comparing element prevalence without initial diversity:

In the first set of simulations, three replicates were taken for dominant, co-dominant, and recessive mutations with varying selection rates (refer to Section 7.2.1 for full conditions). This was examined for a starting element rate of 0.1, 0.5, and 0.9 (results not shown). These results illustrated that at a starting element rate of 0.1, the maternally transmitted element causing PGE never reached fixation. It would be expected that at this starting rate, the element would fix 10% of the time. It was decided to introduce a standing initial diversity rate so some members of the population may already have deleterious alleles to be inherited by their offspring. I could select the percentage of alleles that were initially mutated within the population prior to mating and accelerate the mutation rate to increase chances of haplodiploidy. Without an initial diversity, there is no immediate consequence of recessive deleterious alleles in the population. This better resembles the transition from an initial diploid state with some standing genetic diversity to haplodiploidy.

7.3.2 Comparing element prevalence with initial diversity of 0.01:

In the second set of simulations, 10 replicates were taken for dominant, co-dominant, and recessive strongly deleterious mutations (0.8) (refer to Section 7.2.1 for full conditions). The choice of strongly deleterious mutations was used as this value will likely not be found in nature, and would be a clear indication of whether haploid males becomes fixed in the population. If this did not occur at such an extreme, it is unlikely that the results will be clear around neutrality, where mutations are more likely to occur in the wild. Simulations were performed for a starting element rate of 0.1, 0.5, and 0.9. It was expected that if only genetic drift was acting on neutral mutations (selection coefficient 1.0), mutations would fix at 10%

(for starting element rate 0.1), 50% (for starting element rate 0.5), and 90% (for starting element rate 0.9). Standing genetic diversity was initially set at a low value of 0.01; this illustrated that 1% of alleles in the original population will mutated prior to mating. Results are provided in Table 7.4.

Table 7.4. Expected and observed rates of fixation of the maternally-transmitted cytoplasmic element in 10 replicates for deleterious mutations (0.8) over 100 generations in a population size of 100. Starting element rate varied at 0.1, 0.5, 0.9, with an initial standing diversity rate of 0.01. Fixation rate is what would be expected for neutral mutations under genetic drift.

Conditions	**Expected Fixation Rate**	**Observed Fixation Rate**
Dominant; Starting element rate 0.1	1/10	1/10
Dominant; Starting element rate 0.5	5/10	6/10
Dominant; Starting element rate 0.9	9/10	7/10
Co-dominant; Starting element rate 0.1	1/10	1/10
Co-dominant; Starting element rate 0.5	5/10	3/10
Co-dominant; Starting element rate 0.9	9/10	8/10
Recessive; Starting element rate 0.1	1/10	0/10
Recessive; Starting element rate 0.5	5/10	5/10
Recessive; Starting element rate 0.9	9/10	9/10

Following results from Chapter 6, it was anticipated that the element would spread through the population and evolve the population from diploidy to haplodiploidy. The largest discrepancy was for dominant mutations at 0.9 starting element rate, which illustrated fixation of 70% instead of 90%. This is not a significant difference, but may suggest a slight element disadvantage, since it is fixing at a lower rate than expected. However, the effect may have been too subtle to be seen with this number of replicates or this amount of standing genetic variation. To further explore this possibility, a third set of simulations were performed with an initial standing genetic diversity of 0.1, instead of 0.01.

7.3.3 Comparing element prevalence with initial diversity of 0.1:

The third set of simulations were repeated as in Section 7.3.2, however the initial standing diversity rate was set to a high value of 0.1. This means that 10% of alleles in the original population will already be mutated prior to mating. At this high diversity it is best to have a haplodiploid mating system as the strongly deleterious mutations will be exposed and removed due to the haploid nature of the males. As before, 10 replicates were taken for dominant, co-dominant, and recessive mutations which were strongly deleterious (0.8) with a starting element rate was 0.1, 0.5, and 0.9. All other parameters were as previously outlined. Results of this set of simulations are provided in Figures 7.2-7.4.

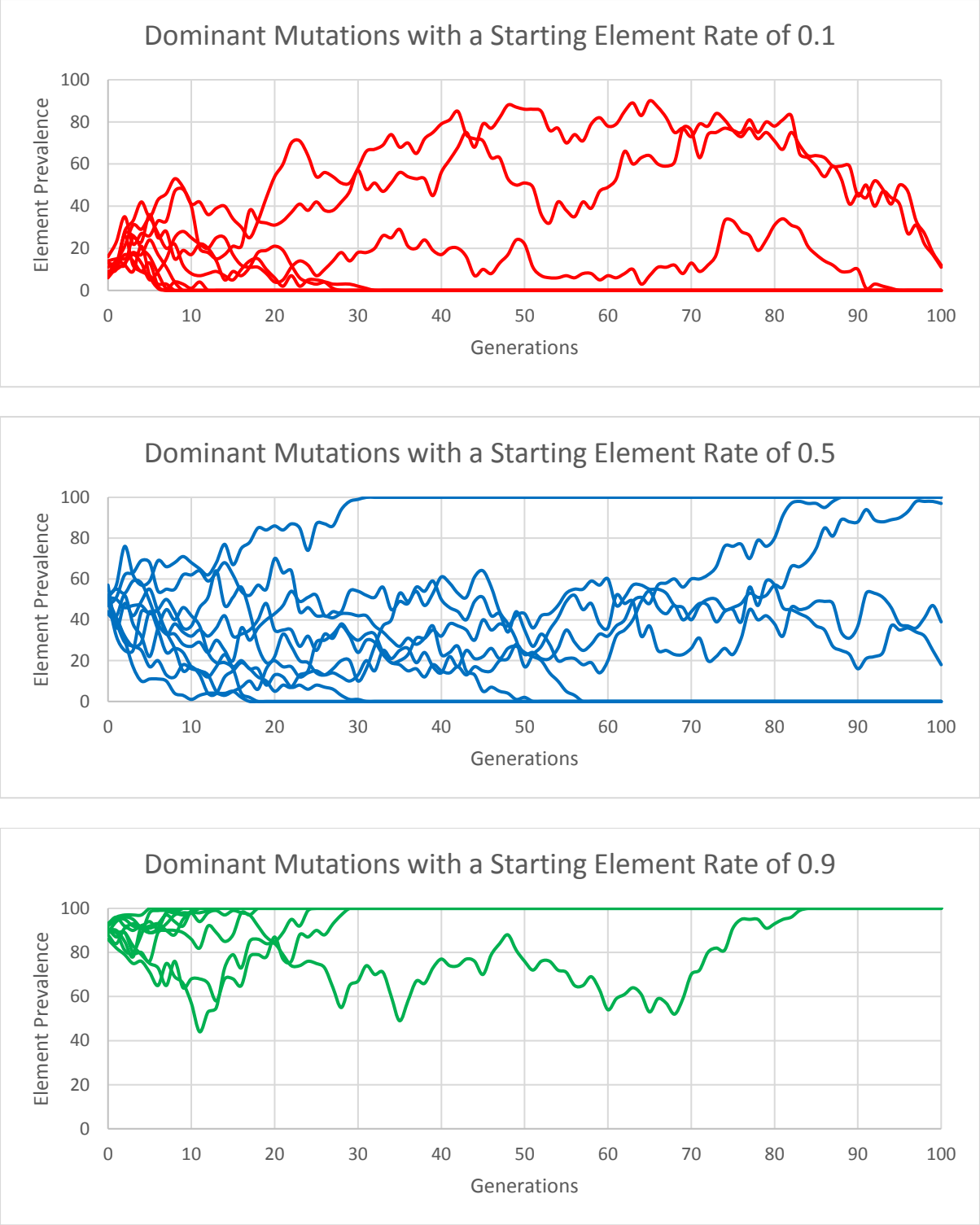


Figure 7.2. Element prevalence after 10 replicates with dominant, strongly deleterious (0.8) mutations over 100 generations in a population size of 100 individuals. Starting element rate was 0.1, 0.5, or 0.9, and the standing diversity rate was 0.1 (10% of alleles in the original population will already be mutated prior to mating). Fixation rates were not significantly different from initial frequencies in any simulation, suggesting that drift alone is responsible for determining the fate of the element.

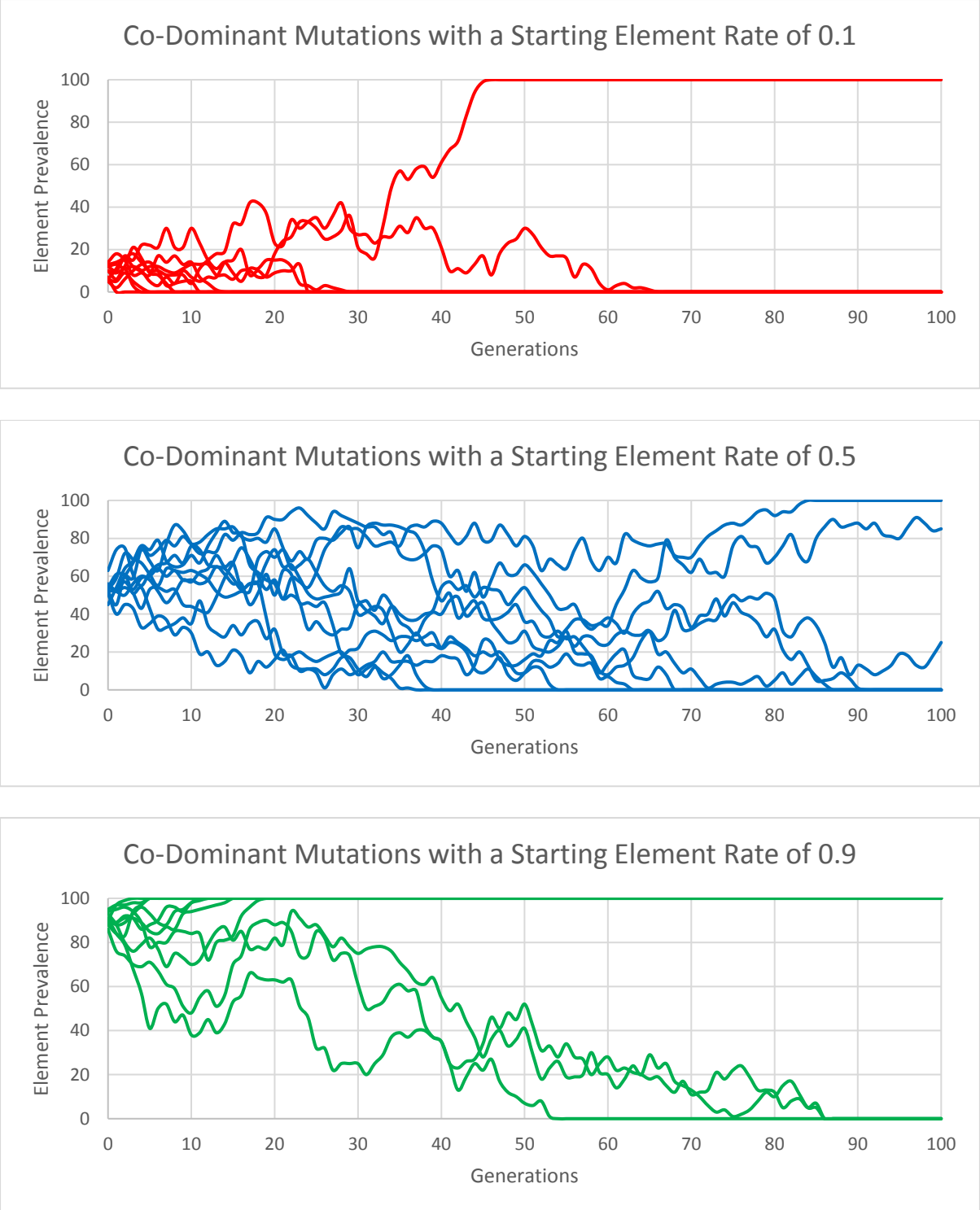
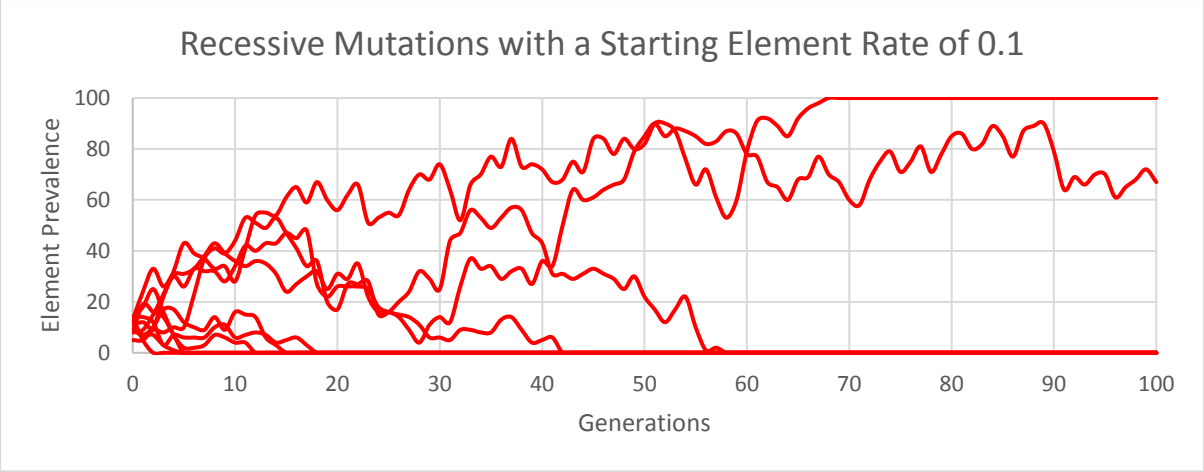
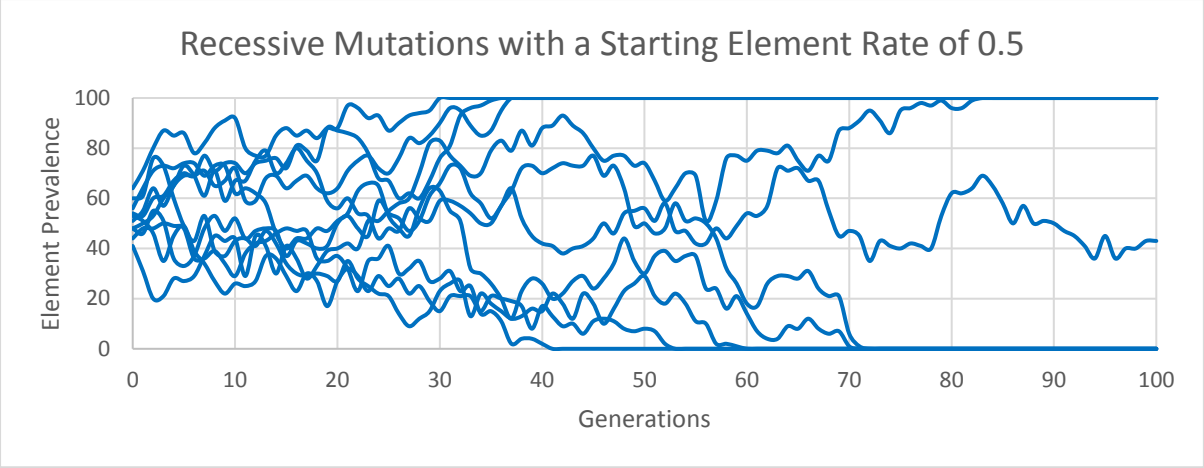


Figure 7.3. Element prevalence after 10 replicates with co-dominant, strongly deleterious mutations over 100 generations in a population size of 100 individuals. Starting element rate was 0.1, 0.5, or 0.9, and the standing diversity rate was 0.1 (10% of alleles in the original population will already be mutated prior to mating). Fixation rates were not significantly different from initial frequencies in any simulation, suggesting that drift alone is responsible for determining the fate of the element.





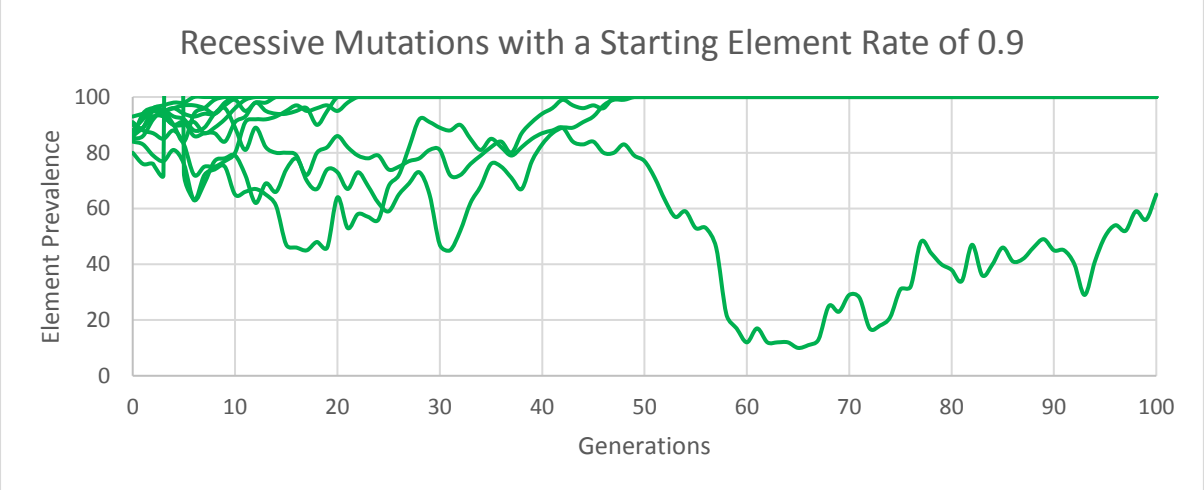


Figure 7.4. Element prevalence after 10 replicates with recessive, strongly deleterious mutations over 100 generations in a population size of 100 individuals. Starting element rate was 0.1, 0.5, or 0.9, and the standing diversity rate was 0.1 (10% of alleles in the original population will already be mutated prior to mating). Fixation rates were not significantly different from initial frequencies in any simulation, suggesting that drift alone is responsible for determining the fate of the element.

In general, Figures 7.2 to 7.4 illustrated levels of fixation for starting element rates of 0.1, 0.5, and 0.9 which would be expected for neutral mutations under genetic drift (fixation at 10%, 50%, and 90% of the time, respectively). A total of 50 replicates were taken (results not shown), and discussed in Table 7.5 to establish whether the results were statistically significant.

Table 7.5. Summary table of 50 replicates, including the results from Figures 7.2-7.4 illustrating the rate of fixation for deleterious mutations (0.8) over 100 generations in a population size of 100 individuals. Starting element rate varied at 0.1, 0.5, 0.9, with an initial standing diversity rate of 0.1.

Conditions	**Expected Fixation Rate**	**Observed Fixation Rate**	**Statistical Significance**
Dominant; Selection 0.8; Starting element rate 0.1	5/50	Fixed: 2/48 Lost: 46/48	0.9597 / 0.1289
Dominant; Selection 0.8; Starting element rate 0.5	25/50	Fixed: 20/43 Lost: 23/43	0.7608
Dominant; Selection 0.8; Starting element rate 0.9	45/50	Fixed: 47/49 Lost: 2/49	0.1200 / 0.9631
Co-dominant; Selection 0.8; Starting element rate 0.1	5/50	Fixed: 3/46 Lost: 43/46	0.8516 / 0.3119
Co-dominant; Selection 0.8; Starting element rate 0.5	25/50	Fixed: 20/40 Lost: 20/40	1.0000
Co-dominant; Selection 0.8; Starting element rate 0.9	45/50	Fixed: 37/46 Lost: 9/46	0.9860 / 0.0364
Recessive; Selection 0.8; Starting element rate 0.1	5/50	Fixed: 5/48 Lost: 43/48	0.5314 / 0.6531
Recessive; Selection 0.8; Starting element rate 0.5	25/50	Fixed: 23/41 Lost: 18/41	0.5327
Recessive; Selection 0.8; Starting element rate 0.9	45/50	Fixed: 42/47 Lost: 5/47	0.6714 / 0.5122

Statistical significance was determined for the number of times the element was fixed in the population, using the sign and binomial two-tailed P test for a starting element rate of 0.5 (online), excluding polymorphic data. This is the chance of observing either “x” or more successes, or “y” or fewer successes in “z” trials. When the starting element rate was 0.1 or 0.9, two values were provided: the chance of observing “x” or more successes / chance of observing “y” or fewer successes in “z” trials. The only result which was statistically significant ($p<0.05$) was for co-dominant deleterious mutations with a starting rate of 0.9 ($p=0.0364$). This

states that the chance of observing 37 or fewer successes out of 46 trials under these conditions is not likely, and therefore there may be an element advantage. Due to the number of replicates and conditions examined, it is reasonable to have one positive result simply by chance. In the other instances, the lower than expected fixation rates may suggest an element disadvantage, however these results are not statistically significant. If the element was favoured which would convert more males to haploids, mutations would be fixed at higher rates higher than what is expected under genetic drift.

7.3.4 Comparing element prevalence at selection extremes:

One final set of simulations were performed to confirm the previous results that the maternally transmitted element which converts diploid males into haploids, is not being favoured as originally expected. For these experiments, strongly deleterious (0.8) and strongly beneficial (1.2) mutations, at the three dominance levels, were modelled with a population size of 100, and over 100 generations. Standing diversity rate was kept at 0.1 (as in Section 7.3.3). Element prevalence was only examining for a starting element rate of 0.5. A total of 50 replicates were taken to determine statistical signficance, and all other parameters remained the same. Results are not graphed, but presented in Table 7.6.

Table 7.6. Summary table of results for 50 replicates, excluding polymorphic data, illustrating the rate of fixation for deleterious mutations (0.8) and beneficial mutations (1.2) over 100 generations in a population size of 100. Starting element rate was 0.5, and the standing diversity rate was 0.1.

Conditions	**Expected Fixation Rate**	**Observed Fixation Rate**	**Statistical Significance**
Dominant; Selection 0.8	25/50	Fixed: 23/43 Lost: 20/43	0.7608
Co-dominant; Selection 0.8	25/50	Fixed: 20/43 Lost: 23/43	0.7608
Recessive; Selection 0.8	25/50	Fixed: 13/40 Lost: 27/40	0.0385
Dominant; Selection 1.2	25/50	Fixed: 14/39 Lost: 15/39	0.1081
Co-dominant; Selection 1.2	25/50	Fixed: 20/41 Lost: 21/41	1.0000
Recessive; Selection 1.2	25/50	Fixed: 22/45 Lost: 23/45	1.0000

As before, statistical significance was determined for the number of times the element was fixed in the population, using the sign and binomial two-tailed P test for a starting element rate of 0.5 (online), excluding polymorphic data. This is the chance of observing either "x" or more successes, or "y" or fewer successes in "z" trials. The only condition which was statistically significant ($p<0.05$) was for recessive deleterious mutations ($p=0.0385$). As before, due to the number of replicates and conditions examined, it is reasonable to have one positive result simply by chance. In all other instances, the element does not fix at the expected rates and it is possible these results are what would be expected under genetic drift. This is surprising given the previous results from Chapter 6, as well as previous research elucidating the benefits of haplodiploidy over diploidy. Therefore, while haplodiploidy may provide a population-level advantage, in this model it does not confer a gene-level or an individual-level advantage that would allow haplodiploidy to successfully invade a population.

As haplodiploid mating systems expose and remove deleterious mutations at a fast rate compared to diploids, overall fitness over time is increased (Mable & Otto, 1998), it would be

expected that the element would be fixed at a higher frequency. This would lead to an overall benefit with haplodiploidy compared to diploidy over many generations. Particularly for beneficial mutations, it would be anticipated that the element would be fixed at a higher frequency, as higher divergence for haplodiploids was seen in Chapter 6 simulations, illustrating the assets of this mating system. Some of the reasons why the element may be declining in frequently may be due to diploids having a higher fitness than haploids if mutations are advantageous. Haploids may have more exposed deleterious alleles, when compared to diploids in a mixed population. In addition, selfing (discussed in Section 7.1.3) provides the same reproductive assurance as haplodiploidy, however it is easy to model aneuploid selfing invading a population. Haplodiploidy is the equivalent to selfing, although it does not invade nearly as easily in the model discussed in this chapter. This may possibly be due to a lack of inbreeding in the diploid / haplodiploid competition model, and provides an opportunity for further research with the addition of an inbreeding coefficient.

7.3.5 Future work:

One of the main caveats of modelling is that many direct assumptions regarding the system will be made, and these will influence the results. Therefore, future work could be performed with different parameters to compare haplodiploid and diploid mating systems (a few of which are outlined below).

a. Sex ratios:

Following the addition of a maternally transmitted element, sex ratios were kept equal (1:1); as the population most likely to evolve is the one that adopts the most evolutionary stable strategy, and equal sex ratios tend to evolve (Maynard Smith, 1992). However a biased sex ratio may be favoured in certain situations, as there are different costs associated with the

production of either sons or daughters (Maynard Smith, 1992). Changing the sex ratio would likely have an impact on whether diploidy remained as the prevailing mating system within the model, or whether haplodiploidy would become the dominant system as more males were converted to haploidy. I would expect that for a highly female-biased sex ratio, there would be a greater chance for the diploid females to carry the element causing paternal genome loss. Therefore, males would quickly gain the element and go from being diploid to haploid. In comparison, if there was a male-biased sex ratio, transmission rates of the element would be lower due to the smaller proportion of females. It would be more likely that the element would be lost or be maintained at low rates within the population.

b. Altering mutations:

Mutations were always assumed to all be beneficial or all be deleterious. Future research could have various selection coefficients, where some mutations could be beneficial and others could be deleterious. In comparison, a system with some mutations acting favourably, others acting unfavourably, and some acting neutrally and therefore having no effect on an organism is more realistic of an actual system. Therefore, the influence of the mutations would then depend on which was the predominant type of mutation, therefore the outcome would be on a case-by-case basis. However, based on the results of Chapter 7, diploidy still prevailed as the main mating system as the introduction of the element did not convert a large proportion of males to haploidy. This was regardless of whether mutations were beneficial or deleterious. Therefore, there may be a small effect of having different types of mutations within one system. It was also assumed that mutations acted independently of each other. This could be expanded upon so that mutations could have an additive effect or act in conjunction with one another. This may be more realistic of real-world mutations, with mutations combining together to have an effect on the individual.

7.4 Conclusions

In conclusion, the results of the haplodiploid and diploid competition were not as originally anticipated. Despite the results of Chapter 6, as well as previous literature describing the benefits of haplodiploidy compared to other mating systems (Mable & Otto, 1998, Otto & Gerstein, 2008, Orr & Otto, 1994), the introduction of a maternally transmitted element which turned diploid males into haploid males did not survive in the population to a great extent. The levels which were seen for this element are the equivalent as to what would be expected for neutral mutations being acted on by genetic drift.

Despite haplodiploidy being the most advantageous mating system for the population or species, introducing it is either neutral or subtly disadvantageous at the individual-level. These results illustrate that in spite of the evidence that haplodiploidy exposes and removes deleterious mutations more efficiently than diploids, as well as accumulates beneficial mutations at a faster rate, it is a very difficult process to initially overcome the barrier between diploidy and haplodiploidy. Once an individual is haplodiploid, it is able to thrive at high rates, however the actual process of becoming haplodiploid is difficult. This explains why so many current mating systems are diploid – organisms have been unable to convert to haplodiploidy despite the advantages of this mating system. As such, it may be beneficial to be haplodiploid as a species, but not advantageous at the individual level.

In this model, if the female had the element, she did not require a mate, providing her with reproductive assurance, and she would produce haploid males. Females always presented as diploid, but males would present as haploid if they inherited the element from their mother which eliminated their paternal genome. These haploid males, provided they were fit enough, could mate in the next generation with diploid females. This raises the question of why

reproductive assurance fails to confer an advantage to the haplodiploid. We anticipate that although the element provides an advantage to the individual female through reproductive assurance, as the element is maternally-transmitted, her infected sons can mate but will not transmit the element to their offspring. This provides an explanation for why haplodiploidy did not out-evolve the diploid population, as there is no advantage of the element in haploid males. Further exploration into this model is necessary to confirm these findings and expand upon the results.

Chapter 8: General Discussion of House Dust Mites and Genetic Systems

8.1 Mites are understudied:

Over the course of my research over the past three years into mite fauna, it has become very obvious that the Acari are an understudied taxa at all levels – from the molecular to the biogeographic. Numbers of terrestrial arthropods, insects, and beetles have been estimated based on the percentage distribution of the world's vascular plant species (402, 500) illustrated 7 million arthropods species, 5.5 million insect species, and 1.5 million beetle species (Stork, 2018). In comparison, there are 93 000 Acari DNA sequences in the GenBank database (Dabert, 2006), however there are only 15 mite and tick genomes published on NCBI GenBank (see Chapter 1) (2017).

A main issue regarding accurate classification of mites arises with taxonomy, largely due to inconsistent nomenclature. Although some taxonomy classifications are trustworthy, the existence of ordinal / subordinal classification is not reliable between mite species (Colloff, 1998). Some other main issues surrounding mites involve their small size, incomplete knowledge regarding distribution, and confusion and misidentification between similar species (O'Connor, 2009, Braig & Perotti, 2009, Perotti et al., 2009). Specialist knowledge is often required to accurately identify and distinguish between species of mites. However, mites are a promising taxon for investigating questions in mating system evolution, and act as an emerging model system for life history evolution.

8.2 Main focuses of mite research:

a. Economic:

The majority of research into mites has been from agricultural (economic) and medical perspectives. Although there is an interest in mite species by taxonomists and some evolutionary biologists, there is minimal collaborative research between these fields. For example, much research has centered on the Phytophagous mites, particularly members of the Tetranychidae (spider mites). These species are crop pests and able to develop resistance to modern insecticide (Whalon et al., 2014). In addition, their genetic system is better studied, with many species undergoing arrhenotokous reproduction (Helle & Bolland, 1967). Detailed reviews of mating systems in the Acari can be found in the following references (Oliver, 1971, Oliver, 1977). In comparison to the Tetranychidae and some other mite species, little is known about the mating system for the HDMs.

b. Medical:

In addition, mites are considered highly relevant due to their medical importance as allergens. For some reviews on HDM allergens see the following references (van Bronswijk & Sinha, 1971, Gøtzsche & Johansen, 2008, Thomas, 2010). Focus has been placed on reducing HDM exposure by allergists and scientists worldwide. A study in the Netherlands illustrated that HDM allergen concentrations was higher with increasing age of the home, higher number of people, absence of floor insulation, carpets, older mattresses, natural ventilation (continuous mechanical ventilation decreases mite concentration), dampness in homes, and higher relative humidity (Van Strien et al., 1994). As such, reducing indoor humidity, using mattress and pillow encasements, weekly washing of bedding materials using hot water (55^{o}C or higher),

replacing carpets, curtains and upholstery, frequent vacuuming of carpets, and freezing soft toys may decrease mite allergen levels (Arlian & Platts-Mills, 2001).

As outlined above, connections have been made between house dust mites and allergens, as there are many known factors which increase mite prevalence in the home. Although information regarding mite allergens was not included in the database, in future this could be expanded upon to potentially increase the number of countries and the number of species. However, it should be noted that the most common species of mites are allergen tested; therefore the less common species which are contaminants within the home would likely not be identified within an allergen study.

c. Forensic:

Arthropods, including mites, have been applied to forensic investigations to estimate post-mortem interval (Goff, 1989). Due to their small size and light weight, they are easily transferred between locations by humans, animals, and corpses (Perotti & Braig, 2009a). Therefore, microsatellite mite DNA could be used for population genetic analysis to link a single mite back to its original colony, and identify whether a crime has been committed in a different location than originally thought (Perotti et al., 2009a). Microsatellites are highly variable in the number of repeats between individuals and therefore allow for discrimination between populations (Kantartzi, 2013). Although mites have the potential to be used for corpse movement analysis, full molecular and taxonomic understanding of the species is required. This already poses a problem with using other organisms for similar aspects of forensic entomology, such as the use of blowflies (Amendt et al., 2004, Amendt et al., 2011). Due to the limited knowledge of mites, more attention and progress needs to be completed before mites can regularly be applied in forensic investigations.

8.3 Summary of findings:

a. Biogeography of mites (Chapters 2 & 3):

To increase the deficient knowledge of HDM fauna, a database of HDM fauna from the literature (1950 to the beginning of 2017) was published with the University of Reading (http://dx.doi.org/10.17864/1947.145) (Farncombe, 2018). The main application of this database was to determine whether a latitudinal diversity gradient (LDG) could be established for HDM fauna. Although the concept of a LDG is well-known, the mechanism behind the gradient or why it exists has not been conclusively determined (Mittelbach et al., 2007). Results suggest that a LDG for HDMs may exist, with country being a highly significant factor.

b. *Dermatophagoides farinae* genetic system (Chapters 4 & 5):

DNA was extracted and optimised from three European populations of *D. farinae* mites, before primers were designed for the CO1 mitochondrial region, the EF1a nuclear region, and two non-coding regions of the nuclear genome. A gene tree of 15 sequences depicting single nucleotide polymorphisms in the CO1 region showed high divergence in one population. It is possible that this population was misidentified as *D. farinae* and is a cryptic species. It is not uncommon for a molecular technique to identify cryptic species which previously had been morphologically identified as the same species (Rubinoff & Sperling, 2004).

c. Mating system simulations (Chapters 6 & 7):

Different mating systems in mites were modelled and compared using Perl 5 under a variety of conditions. The success of a mating system was recorded as the average divergence from ancestor over time. Preliminary results showed haplodiploidy as the most successful mating system by rapidly accumulating beneficial mutations (high divergence), and removing

deleterious mutations (low divergence). This model was expanded with all individuals (males and females) in the population beginning development as diploids, however the introduction of a maternally transmitted element which caused paternal genome loss converted males with the element into haploids. Haplodiploidy did not invade the population as anticipated, indicating that although this mating system is most beneficial for a population, it is not the best system for an individual. The individual is unable to overcome the diploid state to successfully convert to and maintain a haplodiploid state.

It is evident that a main priority for the future of mite research is an overall increase of research into HDM fauna in order to expand the database, as well as to explore genetic differences between populations from a variety of locations worldwide.

8.4 Proposals for future work – Biogeography and genetic system:

a. Latitudinal diversity gradient and cryptic species:

Firstly, a common theme with HDMs is that more research, particularly in tropical locations, is necessary for an accurate understanding of HDM fauna. Although it is clear that a LDG exists for many species, with an increase in different species in the tropics compared to temperate locations, it would be interesting to explore this further with HDMs. One of the contributing factors which may influence whether a LDG exists for HDMs is the possibility of cryptic species.

Although there is such a higher proportion of species in the tropics (approximately two-thirds of all described species), the majority of research into cryptic species have been conducted on temperate organisms (Bickford et al., 2007). As such, further research needs to be performed in tropical rainforests. These species-rich habitats may have unknown high levels of cryptic species (Bickford et al., 2007). However, analysis of the Zoological Record database from 1978 to 2006 for cryptic species in relation to number of described species showed an equal distribution among major metazoan taxa and across biogeographical regions when corrected for species richness and study intensity (Pfenninger & Schwenk, 2007). Therefore, this indicates that the number of cryptic species does not increase in tropical locations (Pfenninger & Schwenk, 2007), however no definitive conclusion has been established. Since HDMs have been understudied, particularly in tropical locations, is it very likely that cryptic species for the mites do exist in these regions, something which would have an effect on the presence of a LDG.

My preliminary CO1 mitochondrial results indicate the possibility of a cryptic species in *D. farinae*. The CO1 mitochondrial region has been used as a DNA barcode to successfully

distinguish between species of tropical Lepidoptera, with 97.9% of 521 examined species having distinctive CO1 barcodes (Hajibabaei et al., 2006). Therefore, this marker has the potential to be successfully used for mites to identify cryptic species, however more populations of *D. farinae* would need to be examined.

The Acari is one of the most underrepresented orders with regards to the range of CO1 diversity data published in GenBank (Arabi et al., 2012). One of the major issues with the molecular work I conducted was the lack of *D. farinae* populations available to me. I contacted multiple groups worldwide by email to try to expand my number of populations, however the majority did not respond, or were unable to provide any colonies for me. I also attempted to find my own colonies through hoover dust from my house, however despite many attempts no mites were found. As a result, I had a very small sample size, and future work would require a better representation of *D. farinae* populations worldwide.

b. Nuclear genome research:

Furthermore, for a more accurate illustration of the *D. farinae* genome, more research into nuclear regions would need to be conducted. Due to time constraints, I was unable to explore different nuclear genes across populations to establish whether amplification would have occurred with diverse primers or at different locations in the genome. Little is known about the molecular diversity of HDMs, including *D. farinae*, therefore any information gained may be able to assist with future research. Advancements in molecular techniques, as well as their increased use, can be helpful with the biogeography of this species. DNA sequencing can be used to date phylogenetic events (Hewitt, 1996), and comparative phylogeography has the ability to examine discrepancies between genetic and phylotypic variation (Bernatchez & Wilson, 1998). Molecular datasets have been used to create phylogenies of Old World frogs

(Wiens et al., 2009), Hylid frogs (Wiens et al., 2006), and Emydid turtles (Stephens & Wiens, 2003), to name a few. If molecular techniques could be used to identify mite species based on mitochondrial and nuclear genes, it may be possible to create a fully inclusive phylogenetic tree of HDM fauna. Once a tree was created, it could then be used to determine locations of mite species, as well as determining when diversification happened and whether these rates were higher near the equator. This would provide support for the LDG for HDMs.

8.5 Proposals for future work – Genetic system and mating system:

a. Advancement of genetic markers:

The advancement of new molecular techniques continually assists in discovering more about a species which could not have been determined without the new technologies. For example, examination of allozymes in eusocial Hymenoptera illustrated that diploid insects have higher heterozygosity than haplodiploid insects, possibly due to smaller effective population size, higher inbreeding, and lower mutation rate in haplodiploids (Hedrick & Parker, 1997). However, the advancement of microsatellite loci illustrates increased variability than what is seen in allozyme loci, due to the higher mutation rate of microsatellites (Hedrick & Parker, 1997). As such, issues which are prevalent with certain genetic markers may be overcome with new markers. This can only help acarologists, due to such limited knowledge of the species. In future, techniques such as next generation sequencing and environmental DNA studies may help identify different mutations in HDMs, and this increase in knowledge of the mite nuclear genome could help detect inbreeding and haplodiploidy in a variety of species.

b. Alternative transmission routes:

I only modelled a maternally-transmitted route for the element resulting in PGE. However, this is not the only mode of transmission which would result in the production of haploid males, and it would be interesting to model different transmission routes. Other forms of genome elimination often involve hybridisation events (Beukeboom & Vrijenhoek, 1998, Burt & Trivers, 2006). In these instances, a genome is temporarily "borrowed" from a related species, and expressed in somatic tissues, before being eliminated from the germline. This form of parthenogenesis is clonal, all-female descent, however, requires the use of a "sperm-donor". In another form of genome elimination, androgenesis, the maternal genome is eliminated and

zygotes only inherit sperm-contributing genes. This mode of reproduction has been identified in two species of ant, four species of clam, and one species of cypress tree. The evolution of androgenesis differs: in ants it has evolved from haplodiploidy, whereas in clams and cypress it has evolved from hermaphroditism (Beukeboom & Vrijenhoek, 1998, Burt & Trivers, 2006).

c. Genetic markers and modelling genome elimination:

Within the Acari, embryonic PGE occurs in the Phytoseiidae and Otopheidomenidae (Mesostigmata) (Nagelkerke & Sabelis, 1998, Gardner & Ross, 2014). Although studies have been conducted on the effect of *Cardinium* and *Wolbachia* bacteria in Phytoseiidae mites (Sourassou et al., 2014) and Tetranychidae mites (Ros et al., 2012), it is possible that there are other unidentified endosymbionts causing PGE in different mite species. As genome elimination provides a two-fold transmission advantage, it is a mystery as to why more species do not adopt this mode of inheritance (Gardner & Ross, 2014).

A mathematical kin selection model was developed for the citrus mealybug, *Planococcus citri*, an organism which undergoes PGE (Gardner & Ross, 2014). This model examined how the degree of inbreeding, mode of sex determination, genomic location, pattern of gene expression, and paternal origin of the lost genome had an influence on the alleles involved in PGE. A diploid population with random mating is assumed, and genome elimination may be autosomal, on sex chromosomes, maternally-activated, or paternally-activated. Genome elimination in the heterogametic sex would result in a biased offspring sex ratio, towards males if genome elimination is maternal, and vice versa. Results illustrated that although there is a transmission advantage for some genes, other genes are disadvantaged from reduced transmission. Inbreeding encourages PGE in the heterogametic sex which can result in death of the population under female heterogamety. However, extinction of the population does not occur

under male heterogamety, due to the effect of sex ratio selection. This provides an explanation for the pattern of PGE in inbred heterogametic males. It should be noted that this is most common under XO than XY inheritance; the Y chromosome potentially inhibits the actions of PGE in males (Gardner & Ross, 2014).

There has been minimal research into the presence of endosymbionts in HDMs, including *D. farinae* (Hubert et al., 2012, Kopecký et al., 2013), therefore further knowledge regarding mite genetic system may lead to identification of additional endosymbionts causing PGE. Additional work into nuclear markers in *D. farinae* could possibly identify haplodizing endosymbionts resulting in PGE in this species.

8.6 Concluding remarks:

My research aimed to address some of the deficits in knowledge of house dust mite fauna. This was conducted through the assembly of a database of HDM publications worldwide, as well as examining the mitochondrial and nuclear regions of one of the most widespread species of HDM, *D. farinae*. In addition, a variety of mating systems found among different species of mites were examined through computer based modelling. This was performed to try to explain the prevalence of diploidy in the majority of taxa, even though haplodiploidy has been shown to be the most efficient system at gaining beneficial mutations and removing deleterious mutations.

Any future applications require more information to accurately depict HDM fauna across the globe. However, this data provides a solid starting point for additional research, and contributes to the currently limited understanding of house dust mites and their genetic systems. As previous discussed, a lot of research has been conducted into Tetranychidae mites due to their economic impact, and it would be useful to examine *D. farinae*, *D. pteronyssinus* and *E. maynei* in more detail as the main species of HDMs. Although there are published genomes for those three species, little is known of their genetic system or mating system. As these three mites have such a large impact on allergens negatively impacting human health, it is surprising how little information is known about these species. If more nuclear genome markers were identified, additional information could be provided regarding their mating system. This could possibly result in the development of a biological control, which could be sprayed in homes and affect mite DNA to reduce their development and survival, and help eradicate them.

Focus should first be placed on discovering more about genetic structure and mating systems of the main HDM species, prior to expanding outwards for a larger picture focus on worldwide

biodiversity. It is likely that many mite species are not true HDMs, and therefore they are not as likely to survive outside their natural habitat. Therefore they may have less of an impact on human health. I think the database is a good starting point for mite biodiversity which can be expanded upon in future. Therefore researchers in each country can determine which species are most prevalent and redirect their research efforts towards these species. This can help with household allergen prevention, and potential future forensic uses of mites. There is currently a huge deficit of research which would need to be filled for mites to be used in forensic applications. Genetic information, such as microsatellite markers, would need to be identified in many mite populations to establish which loci are the most variable between populations. Only if this could be accomplished, would it then be possible to link a single mite with its original population, and therefore linking a corpse or object with its original location as trace evidence.

In conclusion, given mites are an understudied system, any information is a step forwards. There are many avenues to explore, using my research involving a solid HDM database, molecular protocols which are most likely to be successful, and computer modelling of interesting genetic systems in mites. While there is still much to learn, my research has enhanced the limited knowledge of HDMs.

Appendix 1: Regions, Country and References of House Dust Mites

Table 2.2. Biogeographical region, countries and references illustrating the 347 studies collected to generate the mite fauna database. More detailed information can be found in the online database (Farncombe, 2018).

Biogeographical Region	Country	Reference
Afrotropical	Algeria	(Abed-Benamara et al., 1983)
Afrotropical	Kenya	(Gitoho & Rees, 1971)
Afrotropical	Mali	(Rijckaert et al., 1981)
Afrotropical	Mauritius	(Guerin et al., 1992)
Afrotropical	Nigeria	(Somorin et al., 1978, Warrell et al., 1975)
Afrotropical	South Africa	(Ordman, 1971, Sinclair et al., 2010)
Afrotropical	Zambia	(Buchanan & Jones, 1972, Buchanan & Jones, 1974, Mwase & Baker, 2006)
Australasian	Australia	(Colloff et al., 1991, Domrow, 1970, Green & Woolcock, 1978, Green et al., 1986, Morgan et al., 1974, Mulvey, 1973, Tovey et al., 1975, Tovey et al., 2000, Wallace & Vogelnest, 2010, Couper et al., 1998)
Australasian	New Zealand	(Abbott et al., 1981, Andrews et al., 1995, Cornere, 1971, Cornere, 1972, Ingham & Ingham, 1976, Martin et al., 1997, Pike & Wickens, 2008)
Australasian	Papua New Guinea	(Anderson & Cunningham, 1974, Green & Woolcock, 1978, Green et al., 1982)
Indo-Malay	Brunei	(Woodcock & Cunnington, 1980)
Indo-Malay	China	(Chen et al., 2008, Lai, 1988, Rao et al., 2006, Wang et al., 2009, Yu et al., 2015)
Indo-Malay	Hong Kong	(Gabriel et al., 1982)
Indo-Malay	India	(Chaudhury et al., 2013, Dar & Gupta, 1979, Gill & Kaur, 2014, Gupta & Roy, 1975, Kannan et al., 1996, Maurya & Jamil, 1982, Modak, 1991, Modak & Saha, 2002, Modak et al., 1991, Modak et al., 2004, Nath et al., 1974, Nayar et al., 1974, Podder et al., 2006, Podder et al., 2009a, Podder et al., 2009b, Podder et al., 2010, Krishna Rao et al., 1973, Saha, 1997, Sharma et al., 2011, Tripathi & Parikh, 1983)
Indo-Malay	Indonesia	(Baratawidjaja et al., 1998, Fain et al., 1969), Malaysia (Fain & Nadchatram, 1980, Ho, 1986, Ho & Nadchatram, 1984, Ho & Nadchatram, 1985, Mariana et al., 2000, Nadchatram, 2005, Rueda, 1985)
Indo-Malay	Philippines	(Catanghal & Paller, 2012, de las Llagas et al., 2005)
Indo-Malay	Singapore	(Fain et al., 1969, Fain & Feinberg, 1970, Chew et al., 1999, Kuo et al., 1999, Neo et al., 1984)
Indo-Malay	Thailand	(Malainual et al., 1995, Wongsathuaythong & Lakshana, 1972)

Nearctic	Canada	(Fain, 1966, Murray & Zuk, 1979)
Nearctic	Mexico	(Fernández-Duro et al., 2013, Fernández-Duro et al., 2012, Servin & Tejas, 1991)
Nearctic	United States of America	(Arlian et al., 1982, Arlian et al., 1992, Aylesworth & Baldridge, 1985, Babe et al., 1995, Carpenter et al., 1985, De Leon, 1961, De Leon, 1962, Fernández-Caldas et al., 1990, Furumizo, 1975, Hannaway & Roundy, 1997, Haramoto, 1961, King et al., 1989, Klein et al., 1986, Lang & Mulla, 1977, Lang & Mulla, 1978a, Lang & Mulla, 1978b, Massey et al., 2010, Moyer et al., 1985, Mulla et al., 1975, Nadchatram et al., 1981, Nelson & Fernandez-Caldas, 1995, Randall et al., 2003, Sharp & Haramoto, 1970, Smith et al., 1985, Vyszenski-Moher et al., 1986, Wharton, 1970, Woodford et al., 1978, Yoshikawa & Bennett, 1979)
Neotropical	Brazil	(Baqueiro et al., 2006, Binotti et al., 2001a, Binotti et al., 2001b, Binotti et al., 2005, Binotti et al., 2003, da Silva et al., 2005, de Oliveira et al., 1999, de Oliveira et al., 2003, Campos et al., 2010, Demite et al., 2007, Ezequiel et al., 2001, Fain, 1967, Galvão & Guitton, 1986, Medeiros et al., 2002, Nascimento et al., 2017, Rijckaert et al., 1981, Terra et al., 2004)
Neotropical	Barbados	(Pearson & Cunnington, 1973)
Neotropical	Chile	(Artigas & Casanueva, 1983, Franjola & Malonnek, 1995, Franjola & Rosinelli, 1999, Muñoz et al., 1986, Rosas & Casanueva, 1991)
Neotropical	Colombia	(Charlet et al., 1977b, Charlet et al., 1977c, Charlet et al., 1977a, Charlet et al., 1978, Navarro et al., 2008, Sánchez-Medina & Zarante, 1996, Fernández-Caldas et al., 1993)
Neotropical	Costa Rica	(Vargas & Mairena, 1991, Vargas & Smiley, 1994), Cuba (Cao Lopez & Estruch Fajardo, 1981, Cuervo et al., 1983, Dusbábek et al., 1982, Estruch Fajardo et al., 1980)
Neotropical	Ecuador	(Valdivieso et al., 2006, Valdivieso et al., 2009)
Neotropical	Martinique	(Marin et al., 2006)
Neotropical	Panama	(Miranda et al., 2002)
Neotropical	Peru	(Caceres & Fain, 1977, Croce et al., 2000, Villanueva et al., 2003)
Neotropical	Puerto Rico	(Montealegre et al., 1997)
Neotropical	Suriname	(Fain & Van Bronswijk, 1973, Fain et al., 1974)
Neotropical	Venezuela	(Hurtado & Parini, 1987)
Palearctic East	China	(Cai & Wen, 1989, Cui et al., 2004, Jiang et al., 2009, Li et al., 2005b, Shen & Li, 2008, Sun et al., 2013, Sun et al., 2014, Tao & Li, 2006, Wang et al., 2007, Wu et al., 2008, Zhao et al., 2009, Zhu & Zhuge, 2007)

Palearctic East	Japan	(Fain et al., 1974, Ishii et al., 1979, Komatsu et al., 1996, Li et al., 2005a, Oshima, 1967, Oshima, 1970, Sakaki & Suto, 1995a, Sakaki & Suto, 1995b, Suto et al., 1992b, Suto et al., 1992a, Suto et al., 1993, Takaoka et al., 1977a, Takaoka et al., 1977b, Takaoka & Okada, 1984, Takeda et al., 1995, Takeda et al., 1997, Takeda et al., 1998, Takeda et al., 2000, Toma et al., 1993, Toma et al., 1998, Yatani et al., 1984, Miyamoto & Ouchi, 1976)
Palearctic East	Korea	(Cho, 1980, Cho & Houh, 1977, Kang & Chu, 1975, Lee & Cho, 1984, Paik et al., 1992, Ree et al., 1997)
Palearctic East	Taiwan	(Chang & Hsieh, 1989, Oshima, 1970, Sun & Lue, 2000, Tsai et al., 1998)
Palearctic West	Belgium	(Fain et al., 1980, Fain, 1966, de Saint Georges-Gridelet & Lebrun, 1973, de Saint Georges-Gridelet, 1975)
Palearctic West	Croatia	(Macan et al., 2003)
Palearctic West	Czech Republic	(Dusbábek, 1975, Dusbábek, 1995, Samšiňák et al., 1972, Samšiňák et al., 1978, Vobrázková et al., 1979)
Palearctic West	Denmark	(Alani & Haarløv, 1972, Andersen, 1985, Haarløv & Alani, 1970, Hallas & Korsgaard, 1983, Hallas & Korsgaard, 1997, Harving et al., 1993, Korsgaard & Harving, 2005, Korsgaard & Hallas, 1979, Korsgaard, 1979, Korsgaard, 1983, Mosbech et al., 1988, Porsbjerg et al., 2002, Sidenius et al., 2002a, Sidenius et al., 2002b)
Palearctic West	Egypt	(Gamal-Eddin et al., 1982, El-Shazly et al., 2006, El-Sherbiny et al., 2010, Kenawy et al., 2012, Frankland & El-Hefny, 1971, Heikal, 2015, Koraiem & Fahmy, 1999, Morsy et al., 1994, Rezk et al., 1996, Sadaka et al., 2000, Saleh et al., 2013, Saleh et al., 1985, Yassin & Rifaat, 1997)
Palearctic West	Finland	(Stenius & Cunnington, 1972)
Palearctic West	France	(Guy et al., 1972, Lascaud, 1978, Pauli et al., 1997, Penaud et al., 1972)
Palearctic West	Germany	(van Bronswijk & Jorde, 1975, Rijckaert et al., 1981)
Palearctic West	Greenland	(Korsgaard & Hallas, 1979, Porsbjerg et al., 2002)
Palearctic West	Hungary	(Halmai & Szócska, 1983, Halmai, 1984, Sebes et al., 1973)
Palearctic West	Iceland	(Guðmundsson et al., 2008, Hallas et al., 2004)
Palearctic West	Iran	(Amoli & Cunnington, 1977, Hagli et al., 2003, Sepasgasarian & Mumcuoglu, 1979, Soltani et al., 2011)
Palearctic West	Ireland	(Clarke et al., 2016)
Palearctic West	Israel	(Fain et al., 1980, Feldman-Muhsam et al., 1985, Kivity et al., 1993, Mumcuoglu et al., 1999, Spieksma, 1973)

Palearctic West	Italy	(Bigliocchi & Maroli, 1995, Castagnoli et al., 1983, Fain, 1966, Maroli & Mari, 1995, Noferi et al., 1974, Ottoboni et al., 1979, Ottoboni et al., 1983)
Palearctic West	Kazakhstan	(Jagofarov & Galikeev, 1987)
Palearctic West	Lithuania	(Dautartiené, 2001)
Palearctic West	Norway	(Aas & Mehl, 1996, Dotterud et al., 1995, Mehl, 1998, Nafstad et al., 1998)
Palearctic West	Poland	(Aloszko et al., 2004, Henszel et al., 2009, Henszel et al., 2010b, Henszel et al., 2010a, Kosik-Bogacka et al., 2012, Kosik-Bogacka et al., 2010, Horak, 1987, Horak et al., 1996, Piontek et al., 2011, Racewicz, 2001, Solarz et al., 2007, Solarz, 1996, Solarz, 1997, Solarz, 1998, Solarz, 2000a, Solarz, 2000b, Solarz, 2001, Solarz, 2006, Solarz, 2009, Solarz, 2010, Solarz, 2011)
Palearctic West	Russia	(Petrova & Zheltikova, 2000, Pletnev & Dmitrieva, 1977, Zheltikova et al., 2007, Zheltikova et al., 1994)
Palearctic West	Saudi Arabia	(Al-Frayh et al., 1997, Al-Nasser, 2011)
Palearctic West	Spain	(Agratorres et al., 1999, Blasco Sabio et al., 1975, Blasco Sabio & Portus Vinyeta, 1973, Boquete et al., 2006, García Abujeta et al., 1992, Gómez et al., 1981, López-Rico et al., 2000, Pagán et al., 2012, Portus & Soledad Gomez, 1976, García Robaina et al., 1996, Sanchez-Covisa et al., 1999, Sastre et al., 2002)
Palearctic West	Sweden	(Turos, 1979, Warner et al., 1999, Wickman et al., 1993)
Palearctic West	Switzerland	(Fain et al., 1980, Mumcuoglu, 1977, Spieksma et al., 1971)
Palearctic West	The Netherlands	(van Bronswijk et al., 1971, van Bronswijk, 1973, de Boer, 1990, de Boer, 2003, Fain, 1966, van der Lustgraaf, 1978, Rijckaert et al., 1981, Spieksma & Spieksma-Boezeman, 1967, Spieksma, 1971, van der Hoeven et al., 1992, Voorhorst et al., 1967, Wassenaar, 1988, van Bronswijk, 1974)
Palearctic West	Turkey	(Acican et al., 1993, Akdemir & Gürdal, 2005, Akdemir & Soyucen, 2009, Atambay et al., 2006, Aycan et al., 2007, Ciftci et al., 2006, Aykut et al., 2013b, Aykut et al., 2013a, Zeytun et al., 2015, Gülegen et al., 2005, Kalpaklıoğlu et al., 1997, Kalpaklıoğlu et al., 2004, Kilinçarslan, 2012, Rijckaert et al., 1981, Zeytun et al., 2016)
Palearctic West	United Kingdom	(Baker & Swan, 2013, Blythe et al., 1975, Blythe et al., 1974, Brown & Filer, 1968, Carswell et al., 1982, Colloff, 1987, Colloff, 1988, Cunnington & Gregory, 1968, Cuthbert et al., 1979, Gabriel et al., 1982, Hart & Whitehead, 1990, Rao et al., 1975, Sesay & Dobson, 1972, Thind & Clarke, 2001, Walshaw & Evans, 1987, Wraith et al., 1979)

Appendix 2: Perl Mating System Scripts

```
#!/usr/bin/perl
print ("Haplodiploid evolution simulator\n");
#1. Parameters you can change:
$genes = 20; #change this to 20,000 later! Set low to begin with, for speed
$popsize = 100; #note this is census population size, not effective population size
$sexratio = 0.9; #proportion males
$generations = 100; #duration of simulation
$mutationrate = 0.001; #too high, change to something plausible later eg 0.000001
$selection = 0.8; #1 for neutral, 0 for lethal, less than 1 for deleterious, more than 1 for beneficial
$recessive = 0;  #0 is fully dominant, 1 is fully recessive

#2. The filename includes a long random number so you don't accidentally overwrite something you
need
$random= int(rand(1000000));
open (OUTFILE, ">haplodiploid5_$random.txt") || die "can't open outfile";
print OUTFILE "Conditions: $genes genes; population size $popsize; sex ratio $sexratio; generations
$generations; mutation rate $mutationrate; selection $selection; recessivity $recessive\n";

#3. These are useful numbers in the program but they depend on the parameters previously put in
$dominant = (1-$recessive);
$males = int($sexratio * $popsize);
$females = ($popsize - $males);

#4. First set up initial population. A male is represented as a string of numbers, a female as two strings
of the same length

for($m= 0; $m<$males; $m++) {    #for each individual
 for ($g=0; $g<($genes); $g++) {
   $mgenome{$m, $g} = 0;
  }
 $malefitnesses[$m] = 1;
}

for($f= 0; $f<2*$females; $f++) {    #for each individual
  for ($g=0; $g<($genes); $g++) {
   $fgenome{$f, $g} = 0;
  }
  $femalefitnesses[$f] = 1;
}

 $fittestmale = &max(@malefitnesses);
 $fittestfemale = &max(@femalefitnesses);

print OUTFILE "Initial setup: fittest male has fitness $fittestmale and fittest female has fitness
$fittestfemale\n";
print ("\nInitial setup: fittest male has fitness $fittestmale and fittest female has fitness
$fittestfemale\n");

#5. Here we start the evolutionary process: make each generation and monitor it

EVOLVE: for ($gen=0; $gen<$generations; $gen++) {
```

```
  print "Doing generation $gen of $generations\n";
#  print OUTFILE ("$gen\t");

 #6. Build the next generation: first make males
  $m=0;
  while ($m<$males) { #starting with no males
   $rf = int (rand($females));
   if ($femalefitnesses[$rf]>rand($fittestfemale)){
    @egg=&Oogenesis;
    $fitness=1;
    for ($g=0; $g<($genes); $g++) {
      $newmales{$m, $g} = $egg[$g];
    $fitness*= ($selection ** $egg[$g]);
    }
    $newmalefitnesses[$m] = $fitness;
    $m++;
   }
  }

#7 then make females - slightly more complicated as they need a sperm too
 $f=0;
 while ($f<$females) { #starting with no females
 $rm = int(rand($males));
 $rf= int(rand($females));
  if (($femalefitnesses[$rf] > rand($fittestfemale))&&($malefitnesses[$rm]>rand($fittestmale))) {
    $h=($f+$females);
    @egg = &Oogenesis;
    @sperm = &Spermatogenesis;
    $fitness=1;
    for ($g=0; $g<($genes); $g++) {
      $newfemales{$f, $g} = $egg[$g];
      $newfemales{$h, $g}  = $sperm[$g];
      if ($egg[$g] eq $sperm[$g]) {
      $fitness *= ($selection ** $egg[$g]);
      } else  {
      $power = ($recessive* &min($egg[$g], $sperm[$g]) + $dominant * &max($egg[$g],
$sperm[$g]));
      $fitness *= $selection ** $power;
      }
      }
      $newfemalefitnesses[$f] = $fitness;
      $f++;
   }
  }

#8. Now reset everything ready for the next generation
 %mgenome = %newmales;
 %fgenome = %newfemales;
 @malefitnesses = @newmalefitnesses;
 @femalefitnesses = @newfemalefitnesses;
 $fittestmale = &max(@malefitnesses);
 $fittestfemale = &max (@femalefitnesses);

print "generation $gen ended, fittest male now $fittestmale, fittest female now $fittestfemale\n";
print OUTFILE "At generation $gen\n MALE GENOMES:\n";
```

```
  for ($m=0; $m<($males); $m++) {
  print OUTFILE "male $m genome:\t";
 for ($g=0; $g<($genes); $g++) {
 print OUTFILE "$newmales{$m, $g} ";
 }
 print OUTFILE "\n";
  }
  print OUTFILE  " FEMALE GENOMES:\n";
  for ($f=0; $f<($females); $f++) {
  print OUTFILE "female $f genome:\t";
 for ($g=0; $g<($genes); $g++) {
 print OUTFILE "$newfemales{$f, $g} ";
 }
 print OUTFILE "\n";
  }
} #ends a generation

#9. Reports back on screen
$changes = 0;
print "Final results are generation $gen\n MALE GENOMES:\n";
  for ($m=0; $m<($males); $m++) {
   print "male $m genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print "$newmales{$m, $g} ";
  $changes += ($newmales{$m, $g});
  }
  print "\n";
   }
   print " FEMALE GENOMES:\n";
   for ($f=0; $f<($females*2); $f++) {
   print "female $f genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print "$newfemales{$f, $g} ";
  $changes += ($newfemales{$f, $g});
  }
  print "\n";
   }
$sites = ($popsize + $females) * $genes;
$divergence = $changes/$sites;
print "Average divergence from ancestor is $divergence\n";
print OUTFILE "Average divergence from ancestor after $generations generations is $divergence\n";
close OUTFILE;

#6. Subroutines
sub Oogenesis {
 $chrom_two = ($females + $rf); #the mother's other chromosome
 for ($g=0; $g<($genes); $g++) {
  if (rand(1) <0.5){
    @egg[$g] = $fgenome{$rf, $g};
  } else {
    @egg[$g] = $fgenome{$chrom_two, $g};
  }
  if (rand(1)<$mutationrate) {
     print "mutation! in oogenesis at locus $g\n";
     @egg[$g]++;
```

```
 }
 }
@return=@egg;
}

sub Spermatogenesis {
 for ($g=0; $g<($genes); $g++) {
  @sperm[$g] = $mgenome{$rm, $g};
  if (rand(1)<$mutationrate) {
     print "mutation! in spermatogenesis at locus $g\n";
     @sperm[$g]++;
  }
 }
@return=@sperm;
}

sub max {
  @array = @_;
$number = @array -1;
@list = sort(@array);
$highest = $list[$number];
}

sub min {
  @array = @_;
@list = sort(@array);
$lowest = $list[0];
}
```

Figure 6.10. Perl haplodiploid mating system script. See Chapter 6 for applications.

```
#!/usr/bin/perl
print ("Diplodiploid evolution simulator\n");
#1. Parameters you can change:
$genes = 20; #change this to 20,000 later! Set low to begin with, for speed
$popsize = 100; #note this is census population size, not effective population size
$sexratio = 0.9; #proportion males
$generations = 100; #duration of simulation
$mutationrate = 0.001; #too high, change to something plausible later eg 0.000001
$selection = 0.8; #1 for neutral, 0 for lethal, less than 1 for deleterious, more than 1 for beneficial
$recessive = 0;  #0 is fully dominant, 1 is fully recessive

#2. The filename includes a long random number so you don't accidentally overwrite something you
need
$random= int(rand(1000000));
open (OUTFILE, ">diplodiploid5_$random.txt") || die "can't open outfile";
print OUTFILE "Conditions: $genes genes; population size $popsize; sex ratio $sexratio; generations
$generations; mutation rate $mutationrate; selection $selection; recessivity $recessive\n";

#3. These are useful numbers in the program but they depend on the parameters previously put in
$dominant = (1-$recessive);
$males = int($sexratio * $popsize);
$females = ($popsize - $males);

#4. First set up initial population. A male is represented as a string of numbers, a female as two strings
of the same length

for($m= 0; $m<2*$males; $m++) {     #for each individual
 for ($g=0; $g<($genes); $g++) {
   $mgenome{$m, $g} = 0;
  }
 $malefitnesses[$m] = 1;
}

for($f= 0; $f<2*$females; $f++) {     #for each individual
  for ($g=0; $g<($genes); $g++) {
   $fgenome{$f, $g} = 0;
  }
  $femalefitnesses[$f] = 1;
}

 $fittestmale = &max(@malefitnesses);
 $fittestfemale = &max(@femalefitnesses);

print OUTFILE "Initial setup: fittest male has fitness $fittestmale and fittest female has fitness
$fittestfemale\n";
print ("\nInitial setup: fittest male has fitness $fittestmale and fittest female has fitness
$fittestfemale\n");

#5. Here we start the evolutionary process: make each generation and monitor it

EVOLVE: for ($gen=0; $gen<$generations; $gen++) {
  print "Doing generation $gen of $generations\n";
#  print OUTFILE ("$gen\t");

 #6. Build the next generation: first make males
```

```
 $m=0;
  while ($m<$males) { #starting with no males
  $rm = int(rand($males));
  $rf= int(rand($females));
   if (($femalefitnesses[$rf] > rand($fittestfemale))&&($malefitnesses[$rm]>rand($fittestmale))) {
    $j=($m+$males);
    @egg = &Oogenesis;
    @sperm = &Spermatogenesis;
    $fitness=1;
    for ($g=0; $g<($genes); $g++) {
      $newmales{$m, $g} = $egg[$g];
      $newmales{$j, $g} = $sperm[$g];
      if ($egg[$g] eq $sperm[$g]) {
      $fitness *= ($selection ** $egg[$g]);
      } else  {
      $power = ($recessive* &min($egg[$g], $sperm[$g]) + $dominant * &max($egg[$g],
$sperm[$g]));
      $fitness *= $selection ** $power;
      }
      }
      $newmalefitnesses[$m] = $fitness;
      $m++;
   }
  }

#7 then make females
 $f=0;
 while ($f<$females) { #starting with no females
 $rm = int(rand($males));
 $rf= int(rand($females));
   if (($femalefitnesses[$rf] > rand($fittestfemale))&&($malefitnesses[$rm]>rand($fittestmale))) {
    $h=($f+$females);
    @egg = &Oogenesis;
    @sperm = &Spermatogenesis;
    $fitness=1;
    for ($g=0; $g<($genes); $g++) {
      $newfemales{$f, $g} = $egg[$g];
      $newfemales{$h, $g} = $sperm[$g];
      if ($egg[$g] eq $sperm[$g]) {
      $fitness *= ($selection ** $egg[$g]);
      } else  {
      $power = ($recessive* &min($egg[$g], $sperm[$g]) + $dominant * &max($egg[$g],
$sperm[$g]));
      $fitness *= $selection ** $power;
      }
      }
      $newfemalefitnesses[$f] = $fitness;
      $f++;
   }
  }

#8. Now reset everything ready for the next generation
 %mgenome = %newmales;
 %fgenome = %newfemales;
 @malefitnesses = @newmalefitnesses;
```

```
 @femalefitnesses = @newfemalefitnesses;
 $fittestmale = &max(@malefitnesses);
 $fittestfemale = &max (@femalefitnesses);

print "generation $gen ended, fittest male now $fittestmale, fittest female now $fittestfemale\n";
 print OUTFILE "At generation $gen\n MALE GENOMES:\n";
  for ($m=0; $m<($males*2); $m++) {
  print OUTFILE "male $m genome:\t";
 for ($g=0; $g<($genes); $g++) {
 print OUTFILE "$newmales{$m, $g} ";
 }
 print OUTFILE "\n";
  }
  print OUTFILE  " FEMALE GENOMES:\n";
  for ($f=0; $f<($females*2); $f++) {
  print OUTFILE "female $f genome:\t";
 for ($g=0; $g<($genes); $g++) {
 print OUTFILE "$newfemales{$f, $g} ";
 }
 print OUTFILE "\n";
  }
} #ends a generation

#9. Reports back on screen
$changes = 0;
print "Final results are generation $gen\n MALE GENOMES:\n";
  for ($m=0; $m<($males*2); $m++) {
  print "male $m genome:\t";
 for ($g=0; $g<($genes); $g++) {
 print "$newmales{$m, $g} ";
 $changes += ($newmales{$m, $g});
 }
 print "\n";
  }
  print " FEMALE GENOMES:\n";
  for ($f=0; $f<($females*2); $f++) {
  print "female $f genome:\t";
 for ($g=0; $g<($genes); $g++) {
 print "$newfemales{$f, $g} ";
 $changes += ($newfemales{$f, $g});
 }
 print "\n";
  }
$sites = $popsize * $genes * 2;
$divergence = $changes/$sites;
print "Average divergence from ancestor is $divergence\n";
print OUTFILE "Average divergence from ancestor after $generations generations is $divergence\n";
close OUTFILE;

#6. Subroutines
sub Oogenesis {
 $chrom_two = ($females + $rf); #the mother's other chromosome
 for ($g=0; $g<($genes); $g++) {
 if (rand(1) <0.5){
   @egg[$g] = $fgenome{$rf, $g};
```

```
  } else {
    @egg[$g] = $fgenome{$chrom_two, $g};
  }
  if (rand(1)<$mutationrate) {
     print "mutation! in oogenesis at locus $g\n";
     @egg[$g]++;
  }
 }
@return=@egg;
}

sub Spermatogenesis {
 $chrom_two = ($males + $rm); #the father's other chromosome
 for ($g=0; $g<($genes); $g++) {
     if (rand(1) <0.5){
    @sperm[$g] = $mgenome{$rm, $g};
  } else {
    @sperm[$g] = $mgenome{$chrom_two, $g};
  }
  if (rand(1)<$mutationrate) {
     print "mutation! in spermatogenesis at locus $g\n";
     @sperm[$g]++;
  }
 }
@return=@sperm;
}


sub max {
  @array = @_;
$number = @array -1;
@list = sort(@array);
$highest = $list[$number];
}

sub min {
  @array = @_;
@list = sort(@array);
$lowest = $list[0];
}
```

Figure 6.11. Perl diploid mating system script. See Chapter 6 for applications.

```
#!/usr/bin/perl
print ("Recombinant haploid evolution simulator\n");
#1. Parameters you can change:
$genes = 20; #change this to 20,000 later! Set low to begin with, for speed
$popsize = 20; #note this is census population size, not effective population size
$sexratio = 0.5; #proportion males
$generations = 10; #duration of simulation
$mutationrate = 0.001; #too high, change to something plausible later eg 0.000001
$selection = 1.2; #1 for neutral, 0 for lethal, less than 1 for deleterious, more than 1 for beneficial
$recessive = 0;  #0 is fully dominant, 1 is fully recessive

#2. The filename includes a long random number so you don't accidentally overwrite something you
need
$random= int(rand(1000000));
open (OUTFILE, ">haplorecomb_$random.txt") || die "can't open outfile";
print OUTFILE "Conditions: $genes genes; population size $popsize; sex ratio $sexratio; generations
$generations; mutation rate $mutationrate; selection $selection; recessivity $recessive\n";

#3. These are useful numbers in the program but they depend on the parameters previously put in
$dominant = (1-$recessive);
$males = int($sexratio * $popsize);
$females = ($popsize - $males);

#4. First set up initial population. Each male or female is represented as a single array of numbers

for($m= 0; $m<$males; $m++) {     #for each individual
 for ($g=0; $g<($genes); $g++) {
   $mgenome{$m, $g} = 0;
  }
 $malefitnesses[$m] = 1;
}

for($f= 0; $f<$females; $f++) {     #for each individual
  for ($g=0; $g<($genes); $g++) {
    $fgenome{$f, $g} = 0;
  }
  $femalefitnesses[$f] = 1;
}

 $fittestmale = &max(@malefitnesses);
 $fittestfemale = &max(@femalefitnesses);

print OUTFILE "Initial setup: fittest male has fitness $fittestmale and fittest female has fitness
$fittestfemale\n";
print ("\nInitial setup: fittest male has fitness $fittestmale and fittest female has fitness
$fittestfemale\n");

#5. Here we start the evolutionary process: make each generation and monitor it

EVOLVE: for ($gen=0; $gen<$generations; $gen++) {
  print "Doing generation $gen of $generations\n";
#  print OUTFILE ("$gen\t");

 #6. Build the next generation: first make males
  $m=0;
```

```
while ($m<$males) { #starting with no males
$rm = int(rand($males));
$rf= int(rand($females));
  if (($femalefitnesses[$rf] > rand($fittestfemale))&&($malefitnesses[$rm]>rand($fittestmale))) {
for ($g=0; $g<($genes); $g++) {
 if (rand(1) <0.5){
   $newmales{$m, $g} = $fgenome{$rf, $g};
 } else {
   $newmales{$m, $g} = $mgenome{$rm, $g};
 }
 if (rand(1)<$mutationrate) {
    print "mutation! at locus $g\n";
    $newmales{$m, $g}++;
 }
}
   $fitness=1;
   for ($g=0; $g<($genes); $g++) {
     $fitness *= ($selection ** $newmales{$m, $g});
     }
     $newmalefitnesses[$m] = $fitness;
     $m++;
  }
 }

#7 then make females
 $f=0;
 while ($f<$females) { #starting with no females
 $rm = int(rand($males));
 $rf= int(rand($females));
  if (($femalefitnesses[$rf] > rand($fittestfemale))&&($malefitnesses[$rm]>rand($fittestmale))) {
 for ($g=0; $g<($genes); $g++) {
 if (rand(1) <0.5){
   $newfemales{$f, $g} = $fgenome{$rf, $g};
 } else {
   $newfemales{$f, $g} = $mgenome{$rm, $g};
 }
 if (rand(1)<$mutationrate) {
    print "mutation! at locus $g\n";
    $newfemales{$f, $g}++;
 }
}
   $fitness=1;
   for ($g=0; $g<($genes); $g++) {
     $fitness *= ($selection ** $newfemales{$f, $g});
     }
     $newfemalefitnesses[$f] = $fitness;
     $f++;
  }
 }

#8. Now reset everything ready for the next generation
 %mgenome = %newmales;
 %fgenome = %newfemales;
 @malefitnesses = @newmalefitnesses;
 @femalefitnesses = @newfemalefitnesses;
```

```
 $fittestmale = &max(@malefitnesses);
 $fittestfemale = &max (@femalefitnesses);

print "generation $gen ended, fittest male now $fittestmale, fittest female now $fittestfemale\n";
print OUTFILE "At generation $gen\n MALE GENOMES:\n";
   for ($m=0; $m<($males); $m++) {
   print OUTFILE "male $m genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print OUTFILE "$newmales{$m, $g} ";
  }
  print OUTFILE "\n";
   }
   print OUTFILE  " FEMALE GENOMES:\n";
   for ($f=0; $f<($females); $f++) {
   print OUTFILE "female $f genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print OUTFILE "$newfemales{$f, $g} ";
  }
  print OUTFILE "\n";
   }
} #ends a generation

#9. Reports back on screen
$changes = 0;
print "Final results are generation $gen\n MALE GENOMES:\n";
  for ($m=0; $m<($males); $m++) {
   print "male $m genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print "$newmales{$m, $g} ";
  $changes += ($newmales{$m, $g});
  }
  print "\n";
   }
   print " FEMALE GENOMES:\n";
   for ($f=0; $f<($females); $f++) {
   print "female $f genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print "$newfemales{$f, $g} ";
  $changes += ($newfemales{$f, $g});
  }
  print "\n";
   }
$sites = $popsize * $genes;
$divergence = $changes/$sites;
print "Average divergence from ancestor is $divergence\n";
print OUTFILE "Average divergence from ancestor after $generations generations is $divergence\n";
close OUTFILE;

#6. Subroutines

sub max {
  @array = @_;
$number = @array -1;
@list = sort(@array);
$highest = $list[$number];
```

```
}

sub min {
  @array = @_;
@list = sort(@array);
$lowest = $list[0];
}
```

Figure 6.12. Perl haptony mating system script. See Chapter 6 for applications.

```
#!/usr/bin/perl
print ("Clonal haploid evolution simulator\n");
#1. Parameters you can change:
$genes = 20; #change this to 20,000 later! Set low to begin with, for speed
$popsize = 20; #note this is census population size, not effective population size
$generations = 10; #duration of simulation
$mutationrate = 0.001; #too high, change to something plausible later eg 0.000001
$selection = 1.2; #1 for neutral, 0 for lethal, less than 1 for deleterious, more than 1 for beneficial

#2. The filename includes a long random number so you don't accidentally overwrite something you
need
$random= int(rand(1000000));
open (OUTFILE, ">haploclonal_$random.txt") || die "can't open outfile";
print OUTFILE "Conditions: $genes genes; population size $popsize; generations $generations;
mutation rate $mutationrate; selection $selection\n";

#3. These are useful numbers in the program but they depend on the parameters previously put in
$females = $popsize;
$sites = $popsize * $genes;

#4. First set up initial population. Each female is represented as a single array of numbers
for($f= 0; $f<$females; $f++) {     #for each individual
  for ($g=0; $g<($genes); $g++) {
   $fgenome{$f, $g} = 0;
  }
  $femalefitnesses[$f] = 1;
}

 $fittestfemale = &max(@femalefitnesses);

print OUTFILE "Initial setup: fittest female has fitness $fittestfemale\n";
print ("\nInitial setup: fittest female has fitness $fittestfemale\n");

#5. Here we start the evolutionary process: make each generation and monitor it

EVOLVE: for ($gen=0; $gen<$generations; $gen++) {
  print "Doing generation $gen of $generations\n";
#  print OUTFILE ("$gen\t");

#7 Make the next generation females
 $f=0;
 while ($f<$females) { #starting with no females
 $rf= int(rand($females));
   if ($femalefitnesses[$rf] > rand($fittestfemale)){
for ($g=0; $g<($genes); $g++) {
    $newfemales{$f, $g} = $fgenome{$rf, $g};
   if (rand(1)<$mutationrate){
     print "mutation! at locus $g\n";
     $newfemales{$f, $g}++;
  }
}
    $fitness=1;
    for ($g=0; $g<($genes); $g++) {
      $fitness *= ($selection ** $newfemales{$f, $g});
      }
```

```
 $newfemalefitnesses[$f] = $fitness;
 $f++;
 }
  }

#8. Now reset everything ready for the next generation
 %fgenome = %newfemales;
 @femalefitnesses = @newfemalefitnesses;
 $fittestfemale = &max (@femalefitnesses);

print "generation $gen ended, fittest female now $fittestfemale\n";
#print OUTFILE "At generation $gen\n FEMALE GENOMES:\n";
#   for ($f=0; $f<($females); $f++) {
#   print OUTFILE "female $f genome:\t";
#  for ($g=0; $g<($genes); $g++) {
#  print OUTFILE "$newfemales{$f, $g} ";
#  }
#  print OUTFILE "\n";
#  }
 #ends a generation
$divergence = $changes/$sites;
$changes = 0;
print "Final results are generation $gen\n FEMALE GENOMES:\n";
   for ($f=0; $f<($females); $f++) {
   print "female $f genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print "$newfemales{$f, $g} ";
  $changes += ($newfemales{$f, $g});
  }
  print "\n";
   }
   print OUTFILE "$divergence\n";
}

#9. Reports back on screen
$changes = 0;
print "Final results are generation $gen\n FEMALE GENOMES:\n";
   for ($f=0; $f<($females); $f++) {
   print "female $f genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print "$newfemales{$f, $g} ";
  $changes += ($newfemales{$f, $g});
  }
  print "\n";
   }

print "Average divergence from ancestor is $divergence\n";
print OUTFILE "Average divergence from ancestor after $generations generations is $divergence\n";
close OUTFILE;

#6. Subroutines

sub max {
  @array = @_;
$number = @array -1;
```

```
@list = sort(@array);
$highest = $list[$number];
}

sub min {
  @array = @_;
@list = sort(@array);
$lowest = $list[0];
}
```

Figure 6.13. Perl clonal haploid mating system script. See Chapter 6 for applications.

```
#!/usr/bin/perl
print ("Clonal diploid evolution simulator\n");
#1. Parameters you can change:
$genes = 20; #change this to 20,000 later! Set low to begin with, for speed
$popsize = 100; #note this is census population size, not effective population size
$generations = 100; #duration of simulation
$mutationrate = 0.001; #too high, change to something plausible later eg 0.000001
$selection = 1.2; #1 for neutral, 0 for lethal, less than 1 for deleterious, more than 1 for beneficial
$recessive = 0;  #0 is fully dominant, 1 is fully recessive


#2. The filename includes a long random number so you don't accidentally overwrite something you
need
$random= int(rand(1000000));
open (OUTFILE, ">diploclonal_$random.txt") || die "can't open outfile";
print OUTFILE "Conditions: $genes genes; population size $popsize, mutation rate $mutationrate,
selection coefficient $selection\n";

#3. These are useful numbers in the program but they depend on the parameters previously put in
$dominant = (1-$recessive);
$females = $popsize;
$sites = $popsize * $genes*2;

#4. First set up initial population. Each female is represented as a single array of numbers
for($f= 0; $f<$females*2; $f++) {     #for each individual
  for ($g=0; $g<($genes); $g++) {
    $fgenome{$f, $g} = 0;
  }
  $femalefitnesses[$f] = 1;
}

 $fittestfemale = &max(@femalefitnesses);

print OUTFILE "Initial setup: fittest female has fitness $fittestfemale\n";
print ("\nInitial setup: fittest female has fitness $fittestfemale\n");


#5. Here we start the evolutionary process: make each generation and monitor it

EVOLVE: for ($gen=0; $gen<$generations; $gen++) {
  print "Doing generation $gen of $generations\n";
#  print OUTFILE ("$gen\t");


#7 Make the next generation females
 $f=0;
 while ($f<$females) { #starting with no females
   $h = $f+$females;
 $rf= int(rand($females));
 $rh=$rf+$females;
   if ($femalefitnesses[$rf] > rand($fittestfemale)){
for ($g=0; $g<($genes); $g++) {
    $newfemales{$f, $g} = $fgenome{$rf, $g};
   if (rand(1)<$mutationrate){
     print "mutation! Genome $f at locus $g\n";
```

```
    $newfemales{$f, $g}++;
  }
     $newfemales{$h, $g} = $fgenome{$rh, $g};
       if (rand(1)<$mutationrate){
    print "mutation! Genome $f at locus $g\n";
    $newfemales{$f, $h}++;
  }
}
   $fitness=1;
   for ($g=0; $g<($genes); $g++) {
     $power = ($recessive* &min($f[$g], $h[$g]) + $dominant * &max($f[$g], $g[$g]));
     $fitness *= $selection ** $power;
     }
 $newfemalefitnesses[$f] = $fitness;
 $f++;
 }
  }



#8. Now reset everything ready for the next generation
 %fgenome = %newfemales;
 @femalefitnesses = @newfemalefitnesses;
 $fittestfemale = &max (@femalefitnesses);
 $fittestfemale1 = &max (@femalefitnesses1);

$divergence = $changes/$sites;
$changes = 0;

print "Final results are generation $gen\n FEMALE GENOMES:\n";
  for ($f=0; $f<($females*2); $f++) {
    $h = $f+$females;
  print "female $f genome:\t";
    for ($g=0; $g<($genes); $g++) {
       print "$newfemales{$f, $g} ";
       $changes += ($newfemales{$f, $g});
    }
  print "\n";
 # print "female $f genome 2:\t";
 #  for ($g1=0; $g1<($genes); $g1++) {
 #      print "$newfemales1{$h, $g1} ";
 #      $changes1 += ($newfemales1{$h, $g1});
 #   }
 # print "\n";
   }
   print OUTFILE "$divergence\n";
}

print "Average divergence from ancestor is $divergence\n";
print OUTFILE "Average divergence from ancestor after $generations generations is $divergence\n";
print "Average divergence from ancestor is $divergence\n";

close OUTFILE;
```

```
#6. Subroutines

sub max {
  @array = @_;
$number = @array -1;
@list = sort(@array);
$highest = $list[$number];
}

sub min {
  @array = @_;
@list = sort(@array);
$lowest = $list[0];
}
```

Figure 6.14. Perl clonal diploid mating system script. See Chapter 6 for applications.

```
#!/usr/bin/perl
print ("Simulates competing haplodiploids and diploids, difference encoded by cytplasmic element\n");
#1. Parameters you can change:
$genes = 20; #Set low to begin with, for speed
$popsize = 100; #note this is census population size, not effective population size
$sexratio = 0.5; #proportion males
$generations = 100; #duration of simulation
$mutationrate = 0.001; #too high, change to something plausible later eg 0.000001
$diversity = 0.01;
$selection = 1; #1 for neutral, 0 for lethal, less than 1 for deleterious, more than 1 for beneficial
$recessive = 0;  #0 is fully dominant, 1 is fully recessive
$elstart = 0.9; #proportion with the element to begin with

#2. The filename includes a long random number so you don't accidentally overwrite something you need
$random= int(rand(1000000));
open (OUTFILE, ">haplodiploid5_$random.txt") || die "can't open outfile";
print OUTFILE "Conditions: $genes genes; population size $popsize; sex ratio $sexratio; generations $generations; mutation rate $mutationrate; element diversity $diversity; selection $selection; recessivity $recessive; element start $elstart\n";

#3. These are useful numbers in the program but they depend on the parameters previously put in
$dominant = (1-$recessive);
$males = int($sexratio * $popsize);
$females = ($popsize - $males);

#4. First set up initial population as diploids. A male is represented as a string of numbers, a female as two strings of the same length

for($m= 0; $m<$males; $m++) {     #for each individual
 if (rand(1)<$elstart) {
   $melement[$m] = 1;
   $prevalence++;
   for ($g=0; $g<($genes); $g++) {
     if (rand(1)<$diversity) {
      $mgenome{$m, $g} = 1;
     } else {
     $mgenome{$m, $g} = 0;}
    }
   }
 else {
   $melement[$m] = 0;
   $n = $m+$males;
 for ($g=0; $g<($genes); $g++) {
     if (rand(1)<$diversity) {
      $mgenome{$m, $g} = 1;
     } else {
     $mgenome{$m, $g} = 0;}
     if (rand(1)<$diversity) {
      $mgenome{$n, $g} = 1;
     } else {
     $mgenome{$n, $g} = 0;}
  }
 }
```

```
 $malefitnesses[$m] = 1;

} #Some males and some females have have the element; the $melement list is used as a tag for whether the male is haploid or diploid.

for($f= 0; $f<$females; $f++) {     #for each individual
 $h = $f+$females;
  for ($g=0; $g<($genes); $g++) {
   if (rand(1)<$diversity) {
   $fgenome{$f, $g} = 1;
   } else {
   $fgenome{$f, $g} = 0;
  }
    if (rand(1)<$diversity) {
   $fgenome{$h, $g} = 1;
   } else {
   $fgenome{$h, $g} = 0;
  }
  }
  $femalefitnesses[$f] = 1;
  if (rand(1)<$elstart) {

    $felement[$f] = 1;
    $prevalence++;
    }
  else {
    $felement[$f] = 0;
    }
  }
 $fittestmale = &max(@malefitnesses);
 $fittestfemale = &max(@femalefitnesses);

  print "Initial setup, fittest male now $fittestmale, fittest female now $fittestfemale\n";
  print OUTFILE "Initial setup, fittest male now $fittestmale, fittest female now $fittestfemale\n";
 print "At generation $gen\n MALE GENOMES:\n"; #print out whole genome!
   for ($m=0; $m<($males*2); $m++) {
    print  "male $m genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print  "$mgenome{$m, $g} ";
  }
  print  "\n";
   }
    print   " FEMALE GENOMES:\n";
    for ($f=0; $f<($females*2); $f++) {
    print  "female $f genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print "$fgenome{$f, $g} ";
  }
  print  "\n";
   }


print OUTFILE "Initial setup: fittest male has fitness $fittestmale and fittest female has fitness $fittestfemale\n";
print OUTFILE "Element prevalence: $prevalence of $popsize individuals have the element.\n";
```

```
print ("\nInitial setup: fittest male has fitness $fittestmale and fittest female has fitness
$fittestfemale\n");
print "Element prevalence: $prevalence of $popsize individuals have the element.\n";

#5. Here we start the evolutionary process: make each generation and monitor it

EVOLVE: for ($gen=0; $gen<$generations; $gen++) {
  print "Doing generation $gen of $generations\n";
#  print OUTFILE ("$gen\t");
$prevalence = 0;
 #6. Build the next generation: first make males
  $m=0;
  while ($m<$males) { #starting with no males
  $n = $m+$males;
   $rf = int (rand($females)); #choose a mother
   if ($femalefitnesses[$rf]>rand($fittestfemale)){
     @egg = &Oogenesis;
    if ($felement[$rf] eq 0) {
      $newmelement[$m] = 0;
      $rm = int(rand($males)); #choose a male here; you can make HD with or without reproductive
assurance by tweaking this bit
      if ($malefitnesses[$rm]>rand($fittestmale)){ #think carefully: in this simulation the diploids may
have more extreme fitness than the haploids
        @sperm = &Spermatogenesis; #need to tweak subroutine; this will differ between mhaploid and
diploid males
        $fitness = 1;
        for ($g=0; $g<($genes); $g++) {
         $newmales{$m, $g} = $egg[$g];
         $newmales{$n, $g} = $sperm[$g]; #makes a diploid
         if ($egg[$g] eq $sperm[$g]) {
           $fitness *= ($selection ** $egg[$g]);
         } else  {
           $power = ($recessive* &min($egg[$g], $sperm[$g]) + $dominant * &max($egg[$g],
$sperm[$g]));
          $fitness *= $selection ** $power;
     }
     }
     $newmalefitnesses[$m] = $fitness;
     $m++;
       }
    } else { #still with our fitness-checked female, but what if she has the element
    $newmelement[$m] = 1;
    $fitness=1;
    for ($g=0; $g<($genes); $g++) {
      $newmales{$m, $g} = $egg[$g]; #makes a haploid male
    $fitness*= ($selection ** $egg[$g]);
    }
    $newmalefitnesses[$m] = $fitness;
    $m++;
    $prevalence++;
   }
}
} #see if that works...
```

```
#7 then make females - similar to before, only the element has been introduced, maternally
transmitted
 $f=0;
 while ($f<$females) { #starting with no females
 $rm = int(rand($males));
 $rf= int(rand($females));
  if (($femalefitnesses[$rf] > rand($fittestfemale))&&($malefitnesses[$rm]>rand($fittestmale))) {
   $h=($f+$females);
   @egg = &Oogenesis;
   @sperm = &Spermatogenesis;
   $fitness=1;
   for ($g=0; $g<($genes); $g++) {
    $newfemales{$f, $g} = $egg[$g];
    $newfemales{$h, $g} = $sperm[$g];
    if ($egg[$g] eq $sperm[$g]) {
    $fitness *= ($selection ** $egg[$g]);
    } else  {
    $power = ($recessive* &min($egg[$g], $sperm[$g]) + $dominant * &max($egg[$g],
$sperm[$g]));
    $fitness *= $selection ** $power;
    }
    }
    $newfelement[$f] = $felement[$rf];
    $prevalence += $newfelement[$f];
    $newfemalefitnesses[$f] = $fitness;
    $f++;
  }
 }

#8. Now reset everything ready for the next generation
 %mgenome = %newmales;
 %fgenome = %newfemales;
 @melement = @newmelement;
 @felement = @newfelement;
 @malefitnesses = @newmalefitnesses;
 @femalefitnesses = @newfemalefitnesses;
 $fittestmale = &max(@malefitnesses);
 $fittestfemale = &max (@femalefitnesses);

print "generation $gen ended, fittest male now $fittestmale, fittest female now $fittestfemale\n
Element prevalence: $prevalence of $popsize individuals have the element.\n";
print OUTFILE"generation $gen ended, fittest male now $fittestmale, fittest female now
$fittestfemale\n
Element prevalence: $prevalence of $popsize individuals have the element.\n";
#print OUTFILE "At generation $gen\n MALE GENOMES:\n"; #print out whole genome!
   for ($m=0; $m<($males*2); $m++) {
   #print OUTFILE "male $m genome:\t";
  for ($g=0; $g<($genes); $g++) {
  #print OUTFILE "$newmales{$m, $g} ";
  }
  #print OUTFILE "\n";
   }
   #print OUTFILE  " FEMALE GENOMES:\n";
   for ($f=0; $f<($females*2); $f++) {
   #print OUTFILE "female $f genome:\t";
```

```
  for ($g=0; $g<($genes); $g++) {
  #print OUTFILE "$newfemales{$f, $g} ";
  }
  #print OUTFILE "\n";
   }
} #ends a generation

#9. Reports back on screen
$changes = 0;
print "Final results are generation $gen\n MALE GENOMES:\n";
  for ($m=0; $m<($males*2); $m++) {
   print "male $m genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print "$newmales{$m, $g} ";
  $changes += ($newmales{$m, $g});
  }
  print "\n";
   }
   print " FEMALE GENOMES:\n";
   for ($f=0; $f<($females*2); $f++) {
   print "female $f genome:\t";
  for ($g=0; $g<($genes); $g++) {
  print "$newfemales{$f, $g} ";
  $changes += ($newfemales{$f, $g});
  }
  print "\n";
   }
$sites = ($popsize + $females) * $genes;
$divergence = $changes/$sites;
print "Average divergence from ancestor is $divergence\n";
print OUTFILE "Average divergence from ancestor after $generations generations is $divergence\n";
close OUTFILE;

#6. Subroutines
sub Oogenesis {
 $chrom_two = ($females + $rf); #the mother's other chromosome
 for ($g=0; $g<($genes); $g++) {
  if (rand(1) <0.5){
    @egg[$g] = $fgenome{$rf, $g};
  } else {
    @egg[$g] = $fgenome{$chrom_two, $g};
  }
  if (rand(1)<$mutationrate) {
     print "mutation! in oogenesis at locus $g\n";
     @egg[$g]++;
  }
 }
@return=@egg;
}

sub Spermatogenesis {
if ($melement[$rm] eq 0) {  #diploid male
   $chrom_two = ($males + $rm); #the male's other chromosome
  for ($g=0; $g<($genes); $g++) {
  if (rand(1) <0.5){
```

```
  @sperm[$g] = $mgenome{$rm, $g};
  } else {
  @sperm[$g] = $mgenome{$chrom_two, $g};
  }
   if (rand(1)<$mutationrate) {
     print "mutation! in spermatogenesis at locus $g\n";
     @sperm[$g]++;
  }
  }
} else { #haploid male with element
  for ($g=0; $g<($genes); $g++) {
  @sperm[$g] = $mgenome{$rm, $g};
  if (rand(1)<$mutationrate) {
     print "mutation! in spermatogenesis at locus $g\n";
     @sperm[$g]++;
  }
  }
  }
@return=@sperm;
}


sub max {
  @array = @_;
$number = @array -1;
@list = sort(@array);
$highest = $list[$number];
}

sub min {
  @array = @_;
@list = sort(@array);
$lowest = $list[0];
}
```

Figure 7.5. Perl diploid with cytoplasmically inherited maternally-transmitted causing haplodiploidy mating system script. See Chapter 7 for applications.

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