Genomic conflict over reproduction in a booklouse (Psocodea: *Liposcelis*): consequences of a maternally transmitted reproductive manipulator on host ecology and genetics

by

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B.Sc., Simon Fraser University, 2012

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Supervisory Committee

Genomic conflict over reproduction in a booklouse (Psocodea: *Liposcelis*): consequences of a maternally transmitted sex ratio distorter on host ecology and genetics

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Abstract

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Genomic conflict is pervasive in nature and affects a number of fundamental evolutionary processes. Genomic conflict occurs when different genetic entities within a species have different interests in terms of the optimal transmission strategy to future generations, resulting in antagonistic interactions between these elements. When this conflict is over the reproduction strategy within an individual, it can result in sex ratio biases in an individual's offspring. For instance, genomic conflict occurs between maternally transmitted genetic elements (such as female limited chromosomes or cytoplasmic elements) and nuclear elements over the optimal sex ratio of an individual's offspring due to the fact that maternally transmitted elements benefit from a female biased sex ratio (as they are transmitted through the matriline) while nuclear elements benefit from an equal sex ratio. I am investigating a maternally transmitted genetic element in a sexual booklouse, *Lipsocelis* nr. *bostrychophila* (Insecta; Psocodea) that manipulates reproduction such that all females carrying it produce exclusively female offspring. This is expected to affect *L.* nr. *bostrychophila* evolution in a number of ways.

I investigated the ecology of *L.* nr. *bostrychophila* to gain a better understanding of whether and how the selfish reproductive manipulator (designated the distorting element) persists over time. I found that the distorting element is able to persist in *L.* nr. *bostrychophila* populations, both in the wild and in the laboratory, and this is partially due to the fact that females that carry the distorting element have a shorter lifespan and do not produce as many offspring as females that do not carry the element. This helps to counteract the advantage that females carrying the distorting element would otherwise

have due to the fact that they do not produce male offspring. Additionally, I found that females that do not carry the distorting element also produce a female biased sex ratio. This also likely mediates the persistence of the distorting element in wild and laboratory *L.* nr. *bostrychophila* populations, and is particularly interesting in that I found that other wild *Liposcelis* species also exhibit female biased sex ratios. This suggests that *L.* nr. *bostrychophila* populations likely exhibited female bias sex ratios before the distorting element arose in this species.

I also assessed the effect that the distorting element has had on the genomic evolution of *L.* nr. *bostrychophila.* I found that females that carry the distorting element have radically different mitochondria from females that do not carry it, leading me to speculate that the reduced longevity in females that carry the distorting element may be a consequence of impaired mitochondrial function. Finally, I found that all *L.* nr. *bostrychophila* individuals have unusual mitochondria, with females that carry the distorting element having five mitochondrial minichromosomes and females that do not carry the distorting element having seven (rather than the single chromosome typical in animals). These findings contribute to the growing body of evidence suggesting that genomic conflict is an important force shaping species' evolution, supporting the importance of investigating the evolutionary forces at play within as well as between individuals.

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Acknowledgments

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Chapter 1 - Genomic conflict and sex ratios: the genetic battle over host reproduction

Fisher's sex ratio theory (Fisher, 1930) states that an equal ratio of males to females is favoured within a species. The rationale behind this stems from a simple fact: that each individual has one father and one mother. Therefore, if the population sex ratio diverges from 1:1, the rarer sex (and as a consequence individuals that produce more of the rarer sex) will be favoured and these individuals will have a higher fitness until the sex ratio reaches 1:1 again. Although this theory holds for the majority of species, there are also many examples of species that do not have 1:1 sex ratios (Buxton 1941, Hamilton 1967, Owen and Chanter 1969). Further, for some of these species, we know that their skewed population sex ratios are not a temporary condition, but can persist over time (Dyson and Hurst 2004, Perotti et al. 2004). Therefore, why does Fisher's theory not apply to certain species?

In order to investigate this question, we need to consider the assumptions behind Fisher's theory. Several of these assumptions are concerned with the inheritance of genes from parents to offspring and the fact that it must be equal (i.e. from both parents) and "fair" (i.e. Mendelian inheritance) (Bull and Charnov 1988). However, not all genes are inherited by offspring from both their mother and father. Additionally, genes can manipulate the processes involved in sexual reproduction so that they are no longer fair (Rice 2013). Given this, intragenomic conflict, which is concerned with the struggle between genetic entities over their transmission to future generations, is important when considering sex ratio biases in populations.

An introduction to genomic conflict

Genomic conflict is a major force influencing the evolution of species. Genomic conflict occurs as a result of antagonistic interactions between two or more genetic entities over the optimal transmission strategy to future generations (Burt and Trivers, 2006). Genomic conflict is a largely underappreciated player in evolution but despite this, plays a role in many fundamental evolutionary processes. For instance, meiosis and sex

determination, which were traditionally thought to be conserved processes in eukaryotes, are actually surprisingly dynamic. Recent studies have found that genomic conflict is a key factor in the evolution of these processes (Malik and Bayes 2006, Rice 2013, Bachtrog et al. 2014). The importance of genomic conflict in evolution highlights the importance of not only investigating the evolutionary forces at work between individuals or species, but also looking at the evolutionary forces within an individual.

There are many different ways genomic conflict can occur. For instance, genomic conflict can occur between individuals of the same species, within the same individual, and at different times over different processes (Burt and Trivers, 2006). The focus of my thesis is exclusively on genomic conflict occurring within an individual (i.e. intragenomic conflict) over the sex ratio of offspring. In this type of genomic conflict, genetic entities sometimes increase their transmission to future generations by manipulating reproduction to their advantage (i.e. so they are transmitted to a greater proportion of future generations). When this occurs, these elements are called selfish genetic elements (Hurst and Werren 2001). Selfish genetic elements clearly have a negative impact on the genetic element(s) they are in conflict with, and also often have a negative impact on organismal fitness as a whole. Consequently, selfish genetic elements (and genomic conflict) can have a large effect on the evolutionary processes of the species they occur in.

Genomic conflict during reproduction

Although genomic conflict is always present during sexual reproduction, we generally do not observe its effects due to the evolution of the machinery involved in sexual reproduction (for instance meiosis) to keep it "fair" (Malik and Bayes 2006). Additionally, genomic conflict does not always result in a visible change in the population, so it may often go unnoticed. However, in situations where conflict occurs over the sex ratio of offspring, the result of the conflict is often easy to observe (i.e. sex ratio distortion) and so offers a tractable system for investigating the effects of genomic conflict. In addition, conflict over the sex of offspring has several important evolutionary consequences, making it an important field of investigation. For instance, the evolution of novel sex determination systems is proposed in some cases to be the result of genomic

conflict (Engelstädter and Hurst 2006, Beukeboom 2012, Bachtrog et al. 2014). The following are types of genomic conflict that occur during reproduction and can result in sex ratio biases in a population.

Cytoplasmic vs. nuclear conflict

Cytoplasmic vs. nuclear conflict occurs between cytoplasmic elements, which are generally transmitted maternally in the cytoplasm of the egg, and nuclear elements, which are segregated into gametes via meiosis (Hurst 1993). Due to the differences in the transmission of these elements, they have fundamentally different interests in terms of their optimal transmission strategy to the next generation. Specifically, conflict occurs between these elements over the sex ratio of offspring produced by a female. Since cytoplasmic elements (i.e. mitochondria and endosymbiotic microbes in animals) are transmitted maternally, they benefit from an increased investment in female offspring (due to the fact that sons will not pass cytoplasmic elements to their offspring) (Hurst 1993, Engelstädter and Hurst 2009). However, Fisher's sex ratio theory (1930) predicts that nuclear elements should favour an equal investment in male and female offspring. Therefore, conflict occurs between cytoplasmic and nuclear elements over the sex ratio of a female's offspring.

There are numerous examples of selfish cytoplasmic elements that have "won" in their conflict with nuclear elements and manipulate reproduction in an individual (by increasing investment in females) to increase their transmission to future generations. For example, some hermaphroditic plant species contain selfish genetic elements in the mitochondrial genome that increase investment in female reproduction (i.e. ovule production) by causing male sterility (Burt and Trivers, 2006). In animals, there are no known examples of selfish mitochondria but abundant examples of selfish endosymbionts. Endosymbionts (i.e. microbes residing within an organism's body) that manipulate reproduction in their host increase investment in female offspring in three ways (Engelstädter and Hurst 2009). They can feminize genetic males so that they are phenotypically female (feminization) (Rigaud et al. 1992). Endosymbionts can also kill males during development, which increases the amount of resources for the surviving

sisters who transmit the endosymbiont to future generations (male killing) (Jiggins et al. 1998). Finally, maternally transmitted endosymbionts can induce parthenogenesis in their host, which alters the reproduction system of the host so that sexual females become asexual parthenogenetic females (parthenogenesis induction) (Huigens et al. 2000). The common theme for all of these maternally transmitted selfish genetic elements is that they increase the female bias in the offspring of individuals carrying them.

Conflict between homologous elements

Another type of genomic conflict occurring during reproduction is between alternate alleles/chromosomes in a homologous pair. This conflict stems from the fact that there are two alleles of the same gene (or two chromosomes in a homologous pair) that are segregated in meiosis in diploid organisms, and that any allele/chromosome that ends up in more than 50% of an individual's offspring has a transmission advantage to future generations (Burt and Trivers, 2006). For instance, in many females, meiosis is an asymmetric process, with only one of the products of meiosis forming the egg/ovule and the others forming non-viable polar bodies (Fishman and Saunders 2008). Therefore, if an allele can ensure it ends up in the egg/ovule, it benefits. This process, in which a genetic entity sabotages its homologous partner to increase its transmission to future generations, is known as meiotic drive (Hurst and Werren 2001). Female meiotic drive has been reported in several species. For instance, monkeyflowers (*Mimulus guttatus*) have a driving D allele that is transmitted to 58% of the viable ovules in females carrying it (Fishman and Saunders 2008). Understanding conflict between alternate alleles is important for understanding other types of genomic conflict that induce sex ratio biases in a population (for instance, some forms of sex determination conflict). However, as in the example of D alleles in monkeyflowers, there is often no sex ratio bias associated with the selfish genetic element in this form of conflict.

Sex determination conflict

Sex determination occurs in different ways in different taxa (Bachtrog et al. 2014). Many organisms have chromosomal sex determination systems. For instance, in heterogametic sex determination, there are two types of sex chromosomes and the type of

sex chromosomes an individual has determines its sex. In some species the males are the heterogametic sex (i.e. species with XY sex determination) while in others it is females that are heterogametic (ZW species). The number of sex chromosomes an individual has can also determine its sex in some species. For instance, many species have XO sex determination, where females have two X chromosomes and males have one. In still other species it is not sex chromosomes that determine sex but other factors. For instance, in species with haplodiploid sex determination, the ploidy of an individual determines sex, and mothers can either fertilize their eggs to produce diploid females or not fertilize eggs to produce haploid males. However sex is determined, there are many opportunities during sex determination for genomic conflict to occur due to differences in the way the sex is determined in males and females. Conflict over sex determination results in sex ratio biases in an individual's offspring.

In species with XY sex determination, for example, there are several asymmetries in the way sex chromosomes are transmitted to males and females. For instance, the fitness of the Y-chromosome is entirely tied to male function, since these chromosomes are found exclusively in males. Additionally, in males the X-chromosome is transmitted exclusively to daughters. Therefore, genes on the Y-chromosome benefit from a greater proportion of a male's offspring being male, whereas genes of the X-chromosome in males benefit from a greater proportion of female offspring (Rice 2013). Cases of selfish genetic elements on the Y chromosome that cause a male bias are rare (however, see Wood and Newton 1991), perhaps due to the degenerate nature of the Y-chromosome in many species (Rice, 2013). There are, however, many examples of selfish genetic elements on X-chromosomes that cause males to father more female offspring (Jaenike 2001). In these cases, the selfish X-chromosome destroys Y-chromosome bearing sperm so sperm carrying the X-chromosome fertilizes a female's eggs, resulting in these males fathering mostly daughters. As this is a form of meiotic drive, these chromosomes are referred to as driving X-chromosomes. In insects, driving X-chromosomes are found in several Dipteran (fly) species (Jaenike 2001), including several *Drosophila* species and stalk eyed flies (Jaenike 1996, Presgraves et al. 1997).

Conflict between sex chromosomes is similar to conflict between homologous chromosomes in that it is an antagonistic interaction between homologous chromosomes. However, conflict between sex chromosomes is different in that selfish genetic elements on sex chromosomes result in sex ratio biases in a population and sex chromosomes are unlike autosomes in that they are in many cases largely non-recombining (with the exception of the pseudoautosomal region) and generally do not contain the same genetic complement (Bachtrog et al. 2011). Less is known about conflict over reproduction in systems with other sex determination systems. However, one of the most destructive selfish genetic elements, the paternal sex ratio (PSR) chromosome, can be thought of as a selfish genetic element that arose from sex determination conflict (Werren and Stouthamer 2003). This element, which affects some hymenopteran species (that have haplodiploid sex determination), is a supernumerary B chromosome that targets the entire male genetic complement except itself for degradation during embryogenesis (Swim et al. 2012). This chromosome is carried by males and causes these males to father only male offspring due to the fact that PSR destroys paternal autosomal chromosomes (except itself). This results in offspring that would ordinarily be diploid females becoming haploid males, thereby ensuring that the PSR chromosome is transmitted to future generations.

Consequences of selfish reproductive manipulators

I am going to restrict the rest of this discussion to selfish genetic elements that induce a female bias in their host, as these are more common than selfish genetic elements that induce a male bias. When these selfish reproductive manipulators arise in a population, whether they are able to persist over time depends on a number of factors associated with the ecology of their host and the impact they have on the evolution of their host. Additionally, the type of selfish genetic element and its mode of action determine whether it will stably persist in its host over time. Given the number of factors that affect the persistence of selfish reproductive manipulators, it is not surprising that each system containing a selfish reproductive manipulator seems to be slightly different in terms of the frequency of the selfish element found in populations and the

consequences it has on its host. There are, however, a few ways that a wide variety of selfish reproductive manipulators seem to affect their host population.

Many selfish reproductive manipulators induce changes in the mating dynamics in their host population. This is true for both chromosomal and cytoplasmic selfish genetic elements and is likely due to the fact that reproductive manipulators cause sex ratio biases in a population, which results in selection for changes in mating dynamics to cope with the sex ratio bias. For instance, mating preferences often arise against individuals carrying selfish reproductive manipulators. This is the case in populations of the isopod *Armadillidium vulgare*, which harbour a *Wolbachia* endosymbiont that feminizes males (Rigaud et al. 1992). In this system males preferentially mate with females that do not carry the feminizing endosymbiont, which is thought to mediate the negative impact the feminizer has in this system (Moreau et al. 2001). Mating preferences have arisen against individuals carrying selfish reproductive manipulators in several systems, including systems with chromosomal reproductive manipulators such as the stalk eyed fly *Cyrtodiopsis dalmanni*, which contains a selfish X-chromosome (Wilkinson et al. 1998). In addition to the evolution of mating preferences, selfish reproductive manipulators can cause other changes in mating dynamics in their host. For instance, the butterfly *Acraea encedon* carries a male killing *Wolbachia* endosymbiont that causes nearly all males to be killed early in development when they carry the endosymbiont (Jiggins et al. 2002). The presence of the male killing endosymbiont in this system has resulted in a lack of males in *Acraea encedon* populations, which has caused males and females to exhibit reversed gender roles from what is typically observed in butterflies (i.e. female rather than male lekking behaviour to compete for mates) (Jiggins et al. 2000).

In addition to changes in the ecology of their host, selfish reproductive manipulators also often cause changes in host genetics. One of the most well studied ways in which this can occur is through the evolution of genetic suppressors to resist the action of the selfish genetic element. Genetic suppressors have been documented in a number of different systems with a variety of selfish genetic elements (Burt and Trivers, 2006, Jaenike 2001). For instance, in several dipteran systems that contain driving X-

chromosomes, suppressors have arisen on the Y-chromosome (and autosomes) that restore a more equal sex ratio in males carrying the selfish X-chromosome (Presgraves et al. 1997, Jaenike 1999, 2001). The reason that suppressors arise on either the Y-chromosome or autosomes is due to the fact that these genetic entities suffer from the action of the selfish X-chromosome (the Y-chromosome is directly harmed but autosomes are also harmed due to the destruction of half the sperm the male produces) and so these elements are under selection to resist the driving X chromosome's action.

Selfish genetic elements that manipulate reproduction also cause changes in host population genetics. The most common way that this occurs is due to the cotransmission of other genomic elements with the selfish reproductive manipulator. This is most well studied in insects that harbour microbial endosymbionts that manipulate reproduction. Since microbial endosymbionts are maternally transmitted, they are in perfect linkage disequilibrium with all other maternally transmitted elements (ex. mitochondria and female limited W chromosomes) (Hurst and Jiggins 2005). Therefore, the spread of a microbial endosymbiont through a population will result in the incidental spread of all other maternally transmitted elements it is cotransmitted with. For instance, mitochondrial diversity in several insect populations has decreased as a result of selfish microbial endosymbionts, as diverse mitochondrial haplotypes are replaced by the one associated with the reproductive manipulator (Ballard et al. 1996, Jiggins 2003, Shoemaker et al. 2004). The examples above show a few of the most common consequences that selfish reproductive manipulators have on their host populations. However, depending on the interplay between host ecological and genetic factors and the selfish genetic element, a selfish reproductive manipulator can have a variety of effects on its host. Investigating novel systems with different types of selfish genetic elements and different host ecologies is important in the investigation of selfish genetic elements and the effects they have on the ecology and evolution of a species.

A novel insect system containing a maternally transmitted selfish genetic element

Recently, a species of booklouse that harbours a selfish genetic element was discovered (Perlman et al. 2015). Booklice belong to the order Psocodea (formerly the

orders Psocoptera and Phthiraptera), which contains booklice, barklice, and parasitic lice. Booklice are most well known as stored grain pests, and most studies conducted on this group of insects are therefore aimed at reducing their human impact. The focus of my thesis is *Liposcelis* nr. *bostrychophila,* a wild booklouse species originally collected in the Chiricahua Mountains, Arizona, in 2010. This species is sexual, but is interesting in that it contains two distinct female reproductive phenotypes. Some females, designated distorter females, carry a selfish genetic element (the distorting element) and produce exclusively female offspring, while other females, designated normal females, do not carry the selfish genetic element and produce a mixed sex ratio. The distorting element is maternally transmitted and all offspring of a distorter female also carry the distorting element. Additionally, both females are sexual in that they must mate with males to reproduce and incorporate genetic information from males into their offspring (Perlman et al. 2015).

Since the selfish genetic element is maternally transmitted, we originally expected it to be a microbial endosymbiont as these are common maternally transmitted selfish genetic elements in insects (Engelstädter and Hurst 2009). Extensive genomic sequencing and EM imaging, however, has not produced any evidence that the selfish genetic element is a microbial endosymbiont. Therefore, the distorting element is likely some other maternally transmitted element, such as a selfish mitochondrial element or a chromosomal element. There is currently no evidence to suggest the distorting element is mitochondrial. However, genomic sequencing has revealed that part of the nuclear genome in distorter females is not found in other individuals in the population (Perlman et al. 2015). Therefore, the distorting element may be a nuclear element. This is an intriguing finding since no cases of maternally transmitted chromosomal elements that cause sex ratio distortion have been uncovered. In addition, since *Liposcelis* species have XO sex determination, it is difficult to say what type of chromosomal element would be able to cause the sex ratio distortion we see in distorter females (Jostes, 1975). Finally, very little is known about the effects of the distorting element on *L.* nr. *bostrychophila* populations, including whether and how the distorting element is able to persist over time. I employed a combination of experimental, genetic, and field investigations to learn

more about the distorting element in *L.* nr. *bostrychophila* populations, including whether and how it is able to persist and what effect it has on host population genetics and evolution.

Research focus and importance

My thesis is divided into three areas of investigation. In the first section, I investigate the ecology of *L.* nr. *bostrychophila* in laboratory settings to gain more information about how and whether the distorting element persists over time. I performed a number of experiments to determine what effect the distorting element has on individuals that carry it, the reproductive behaviour of individuals that do not carry the distorting element, and how the frequency of the distorting element changes in population cages over time. In the second section of my thesis, I sequence the mitochondrial genome of normal and distorter *L.* nr. *bostrychophila* females to determine how the distorting element has influenced mitochondrial evolution in this species (and to more conclusively rule out the possibility that the distorting element is mitochondrial). However, whether the distorting element is mitochondrial or nuclear, mitochondrial population genetics are likely affected by the distorting element since the distorting element and mitochondria are both transmitted maternally and so are in perfect linkage. Finally, in the last section I explore the species and genetic diversity of wild *Liposcelis* specimens collected four years after the original *L.* nr. *bostrychophila* individuals were collected. The aim of this field collection was to establish whether the distorting element was able to persist in wild populations over time and determine whether the mitochondrial diversity is similar in laboratory and field collected individuals. Additionally, I wanted to investigate other wild *Liposcelis* species, since little is known about the species diversity or genetic diversity of wild *Liposcelis.*

This research provides an in-depth examination into the effects of a maternally transmitted sex ratio distorting element on its host population. *Liposcelis* nr. *bostrychophila* is unique since there are few insects systems in which a species with an XO sex determination system has been found to have a maternally transmitted selfish genetic element that causes individuals to produce exclusively female offspring.

Therefore, information gained about this system will be valuable in contrasting the effects of different selfish genetic elements in hosts with different reproductive systems. Additionally, our information about selfish genetic elements that manipulate reproduction largely comes from a few established systems, often from well-studied insect lineages. Therefore, information from a non-model system in which little is known about the host ecology is exciting, as it provides an example of what sorts of systems may exist in the wild that we have yet to discover.

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Chapter 2 - Female fitness differences and facultative sex allocation mediate the persistence of a selfish genetic element that manipulates reproduction in a booklouse (*Liposcelis* nr. *bostrychophila*)

Abstract

Selfish genetic elements that manipulate host reproduction often have a large ecological and evolutionary impact on their hosts, the degree of which is dependent on a number of genetic and ecological factors. A selfish genetic element was recently discovered in a species of booklouse: *Liposcelis* nr. *bostrychophila*. This selfish genetic element is transmitted with 100% efficiency from mother to offspring, is not microbial, and causes females carrying it to produce exclusively female progeny. I am investigating the ecology of *L.* nr. *bostrychophila,* with the aim of identifying what ecological factors may be important in the persistence of this selfish genetic element. I found that females carrying the selfish genetic element do not live as long and do not produce as many offspring as individuals not carrying the element. Additionally, I found that females that do not carry the selfish genetic element also produce a female biased sex ratio. Finally, populations containing individuals harbouring the selfish element have a very low frequency of males and can have extreme variation in the frequency of the selfish genetic element without causing population collapse. These results suggest that the population dynamics of *L.* nr. *bostrychophila* are complex. However, the lower fitness of females carrying the selfish element as well as the female biased sex ratio produced by females not carrying the selfish element contribute to the persistence of this selfish genetic element in *L.* nr. *bostrychophila* by mediating the advantage females carrying the selfish genetic element otherwise have over other females due to the exclusively female sex ratio they produce.

Introduction

Selfish genetic elements have a large impact on the ecology and evolution of their host species. They are implicated in the evolution of many fundamental biological processes including sex determination (Hurst and Werren 2001, Bachtrog et al. 2014),

sexual conflict (Price and Wedell 2008) and meiosis (Fishman and Willis 2005, Malik and Bayes 2006). Selfish genetic elements increase their transmission to the next generation at the expense of other genomic elements that do not share their evolutionary interests (and possibly at the expense of organismal fitness) (Rice 2013). In some cases this is accomplished through manipulating reproduction in their host to increase their representation in the future generations. There are two main categories of selfish genetic elements that manipulate host reproduction: cytoplasmic elements (such as endosymbionts or organelles) and elements encoded in the nuclear genome (such as selfish sex chromosomes or supernumerary B chromosomes). A common example of cytoplasmic elements that manipulate host reproduction are endosymbiotic bacteria (or other endosymbiotic microbes) that are maternally transmitted in the cytoplasm of the egg and so benefit from causing an increased investment in female offspring in their host (since males do not transmit the element to their offspring) (Engelstädter and Hurst 2009). Nuclear genes, often on sex chromosomes, also can induce sex ratio distortion in their hosts. For instance, selfish sex chromosomes use meiotic drive to bias the sex ratio of their host (Jaenike 2001). Additionally, other nuclear elements such as B chromosomes (i.e. supernumerary chromosomes) can manipulate reproduction in their host (Burt and Trivers 2006).

Several factors are generally taken into consideration when predicting the evolutionary trajectory of a selfish reproductive manipulator (Werren 1987, Hatcher et al. 1999). Some genetic factors that are important are the sex determination system in the host species, the type of reproductive manipulator (i.e. cytoplasmic vs. chromosomal), and the transmission efficiency of the reproductive manipulator to the next generation. There are also several important ecological factors, including the fitness of individuals carrying the element relative to those that do not, the sex ratio produced by hosts that do not carry the selfish genetic element, and the mating dynamics of individuals in the population. The interplay between these factors determines the long-term stability of selfish genetic elements that manipulate reproduction within a population. Although the population dynamics of selfish reproductive manipulators in their host have been investigated in several systems (Jiggins et al. 2000, Price et al. 2014), the systems used

are often very different from each other in many ways and empirical and theoretical studies often do not come to the same conclusions concerning the stable frequency of selfish genetic elements in populations (Kelly et al. 2001). Therefore, there is still much to explore in this field of research, particularly using new systems that have not previously been investigated.

One important factor determining the population consequences of a selfish reproductive manipulator in its host is the nature of the element itself. In animals, endosymbiotic bacteria (and other microbial endosymbionts) often manipulate reproduction in their host to increase the female bias in the population. There are several ways in which they do this including feminizing genetic males so that they are phenotypically female, killing males, or inducing parthenogenesis in their hosts (Hurst 1993, Engelstädter and Hurst 2009). Selfish sex ratio distorting chromosomes also have several modes of action (Jaenike 2001). For instance, selfish X chromosomes cause males carrying them (in XY species) to produce predominantly female offspring by destroying Y chromosome bearing sperm (Rice, 2013). Additionally, a well-known example of a selfish chromosome in haplodiploid species (i.e. species in which males are haploid and develop from unfertilized eggs while females are diploid and develop from fertilized eggs) is a supernumerary B chromosome called PSR (paternal sex ratio). PSR manipulates reproduction by targeting all of the paternal chromosomes it is transmitted with in males for destruction, causing diploid females to become haploid males (Werren and Stouthamer 2003, Swim et al. 2012). As the two previous examples make clear, the sex determination system in a species is an important factor in the successful invasion of a selfish reproductive manipulator into the population, which is likely why species with certain sex determination systems (such as haplodiploid insects) seem to be more prone to reproductive manipulation by selfish genetic elements (Engelstädter and Hurst 2009, Werren 2011). Additionally, selfish genetic elements have also been proposed to affect the evolution of host sex determination, suggesting feedback between genomic conflict (and selfish genetic elements) and sex determination (Kageyama et al. 2012).

Insects contain a wide variety of selfish genetic elements that manipulate reproduction in their host and contain examples of both chromosomal and cytoplasmic selfish genetic elements in different lineages (Jaenike 2001, Engelstädter and Hurst 2009). The insect booklouse genus *Liposcelis* (Insecta: Psocodea) is a free living lineage of the order Psocodea (containing parasitic lice, booklice, and barklice) that has an XO sex determination system (Nokkala and Golub 2002; Jostes 1975). In species with XO sex determination females have two X chromosomes and males have one. A species within this genus (*L.* nr. *bostrychophila*) was recently discovered and found to harbour a selfish genetic element that manipulates reproduction in its host (referred to as the distorting element) (Perlman et al. 2015). Females that carry the distorting element produce only female offspring who also carry the distorting element (referred to as distorter females) while females that do not carry the distorting element produce a mixed sex ratio (referred to as normal females) (Figure 2-1). Thus, the distorting element is maternally transmitted with 100% efficiency. Additionally, this species is obligately sexual, with distorter females inheriting alleles from both their mother and father (i.e. distorter females are not sperm parasites) (Perlman et al. 2015).

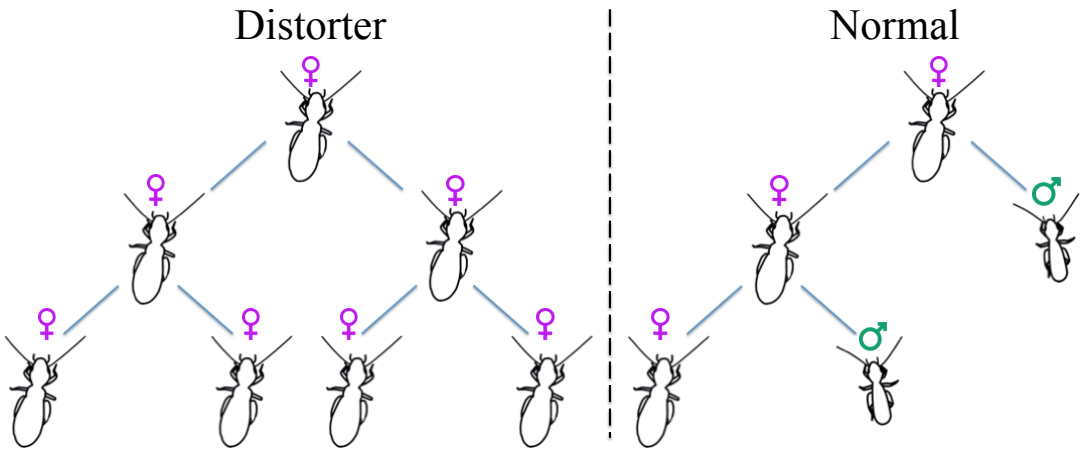

Figure 2-1. Reproductive asymmetry between distorter and normal *L.* nr. *bostrychophila* females. Distorter females avoid the cost of producing males and so have a higher reproductive potential than normal females. In the last generation depicted, distorter females outnumber normal females and there are fewer males than females. Note, however, that this is based on the assumption that both female types produce the same amount of offspring and that normal females produce offspring with a 1:1 sex ratio.

The population dynamics in this system are superficially similar to the effect a feminizing endosymbiont may have on its host. However, extensive genomic sequencing and microscopy has not produced any evidence that *L.* nr. *bostrychophila* harbours any endosymbionts (Perlman et al. 2015). Alternatively, we found that part of the nuclear genome in distorter females is exclusive to these females, suggesting that the distorting element in this system may be a nuclear entity. We still know little, however, about the mechanism of sex ratio distortion in distorter females. I am interested in exploring the ecological consequences of carrying the distorting element in *L.* nr. *bostrychophila* individuals, with the aim of gaining more information about how this selfish genetic element persists. Investigating this system can provide insight into the effect a selfish genetic element that manipulates reproduction has on its host in a novel insect system. This will provide more information about the ecological effects and conditions that enable the persistence of a selfish element that induces a female biased sex ratio in its host.

As stated, the transmission efficiency of a selfish reproductive manipulator to the next generation is an important factor that affects the persistence of the selfish genetic element. For instance, for maternally transmitted elements, there is a large difference in terms of the population consequences and the stable equilibrium frequency of a maternally transmitted selfish genetic element if it causes 51% as opposed to 100% of its host's offspring to be female. This is because, in these types of systems, there is an asymmetry in the reproductive potential of hosts carrying the selfish genetic element and those not carrying it and the magnitude of this asymmetry affects the persistence of the selfish element. This is due to the fact that those carrying the element avoid, to some extent, the cost of producing males (assuming that the selfish element is not a male killer) (Maynard Smith 1978). The cost of producing males (i.e. that sexual species halve their reproductive potential due to the fact that males do not bear offspring themselves but fertilize females' offspring) is often used to contrast the costs sexual vs. asexual reproduction. However, it also applies in systems in which a selfish genetic element induces a female biased sex ratio in its host, since some females produce more female offspring than others and they are all mating with the same pool of males. Theoretical

studies suggest that, all other factors being equal, in systems in which the transmission of a selfish genetic element inducing a female bias is high, the element is expected to increase in frequency, rapidly leading to an inherently unstable system due to male limitation (Werren 1987, Hatcher et al. 1999). Therefore, in systems in which selfish genetic elements causing a female bias persist over time, "all other factors" must not be equal and it is likely that ecological factors allow for the persistence of the selfish element.

Often in populations with selfish genetic elements that manipulate reproduction, there are fitness differences between those carrying the element and those not. These fitness differences affect the stable frequency of the selfish genetic element in the population. The relative fitness differences can be in either direction between hosts carrying the element and those not. For instance, in the amphipod *Gammarus duebeni* there is a cost to carrying a microsporidian-feminizing agent that results in a lower fecundity in those infected with the feminizer compared to those that are not (Kelly et al. 2001). In contrast to this, some whiteflies (*Bemisia tabaci*) carry a *Rickettsia* endosymbiont that causes them to produce offspring that have higher survival during juvenile stages and higher fecundity than those that do not carry it. This fitness benefit is also associated with a higher proportion of female offspring in individuals carrying *Rickettsia*, facilitating the spread of *Rickettsia* through whitefly populations in Arizona (Himler et al. 2011). The direction and strength of relative fitness differences between hosts carrying the sex ratio distorting selfish genetic element and those not carrying it are important predictors of the long term persistence of the selfish genetic element (Werren 1987).

Another important ecological factor in systems with selfish reproductive manipulators is the reproductive behaviour of females not carrying the selfish genetic element. The sex ratio that these females produce, as well as whether they can adjust the sex ratio of their offspring in an adaptive manner (i.e. exhibit facultative sex allocation), is expected to influence the persistence of selfish genetic elements that manipulate reproduction (Hatcher and Dunn 1995). It is proposed that facultative sex allocation may

occur whenever one sex has a higher reproductive value than the other sex (West et al. 2002). Much of the work on facultative sex allocation in insects has focused on hymenopterans, since due to their haplodiploid sex determination, mothers have control over the sex of their offspring. In hymenopterans, sex allocation theory often corresponds well to empirical observation (Shuker and West 2004, Raja et al. 2008). However, other systems in which the mechanism by which females control the sex of their offspring is uncertain also exhibit facultative sex allocation, including species that have chromosomal sex determination systems (Avilés *et al.* 2000; Ross *et al.* 2010a). For instance, Seychelles warblers (*Acrocephalus sechellensis*) alter the sex ratio of their broods in response to habitat quality, as females are of higher reproductive value than males in high quality habitats and vice versa in low quality habitats (Komdeur 1996). Seychelles warblers, however, have a ZW sex determination system (i.e. males are ZZ and females are ZW) so the way in which mothers are able to alter the sex of their progeny is uncertain. In *L. nr. bostrychophila*, we have very little information on the reproductive behaviour of normal females. Additionally, it is unknown whether *Liposcelis* species (which have an XO sex determination system) are able to alter the sex of their offspring to suit environmental conditions.

Finally, males can become limited in populations containing selfish genetic elements that cause a female bias in populations, leading to an increased importance of male mating dynamics (Hatcher et al. 1999). Male limitation in these systems is proposed to drive the evolution of a number of factors including an increase in male mating capacity (Moreau and Rigaud 2003), or male mating preferences in which males preferentially mate with females that do not carry the selfish genetic element (Moreau et al. 2001). These factors may be important in *L.* nr. *bostrychophila* populations, and although I am not specifically assessing male mating behaviour in this study, I am assessing the frequency of males in populations, which should provide some information about whether male mating behaviour may be important in this system.

I conducted a series of experiments to learn more about the ecology of *L.* nr. *bostrychophila*. I measured the lifetime fecundity, adult longevity and development time

of normal and distorter females to investigate whether there are fitness differences between the female types. I also assessed the offspring sex ratio normal females produce and whether they alter the sex ratio of their offspring in response to ecological conditions. Finally, I conducted a long-term assessment of the frequency of distorter and normal females in population cages starting with two initial proportions of distorter to normal females. I wanted to assess whether distorter females (and also the distorting element) are able to invade and reach a stable frequency in populations as well as to assess whether populations starting at different initial frequencies would reach the same frequency of distorter females over time (i.e. a stable population frequency). In undertaking this study, my aim was to better understand how the ecology of *L.* nr. *bostrychophila* allowed the distorting element that resides in distorter females to invade and persist in natural populations. But in addition to this, very little is known about the ecology of *Liposcelis* species in natural settings (i.e. excluding species that are stored grain pests) and so a second goal was to increase our knowledge of this insect group. Finally, this system appears to be unique in that to our knowledge there are few documented examples insects with XO sex determination systems harbouring maternally transmitted elements that manipulate reproduction with 100% efficiency. Thus, investigating this species can provide us with more information about selfish genetic elements that manipulate reproduction in their host, including under what conditions this type of sex ratio distortion may exist.

Methods

Colony Information

Liposcelis nr. *bostrychophila* used in this study were collected from the Chiricahua Mountains, Arizona in 2010. Cultures are maintained at 27°C and 75% RH (using a saturated NaCl solution). I maintain distorter female and normal female cultures separately in 125ml glass jars containing a 1:10 (by weight) mixture of Rice Krispies (Kellogg's) and organic cracked wheat (Bob's Red Mill). Since distorter females need to mate with males in order to reproduce I added approximately 25 males from the normal female colonies into the distorter female colonies weekly. Male *Liposcelis* can be distinguished from females based on several morphological characteristics including size

and external reproductive characteristics (Mockford 1993). Unless otherwise stated, I used a 1:10 (by weight) mixture of Rice Krispies to cracked wheat as food in experiments.

Fitness differences between normal and distorter females

I undertook two experiments to examine fitness differences between distorter and normal females: one addressing the development time and longevity of the female types and the other addressing fecundity. The results of the experiment addressing the longevity of normal and distorter females have been published Perlman *et al*. (2015). For the longevity and development time assay I placed 10 eggs laid by age matched and mated females into a small petri dish (35mm in diameter) with 0.7g of food. I produced 10 replicate petri dishes for each female type. When individuals in these containers completed development I recorded the date on which each individual became an adult as well as the sex of the individual. I discarded males and transferred females into a new container, keeping females that had been raised together in the same container as adults. Since I was unable to sex individuals until they developed, I only included individuals that completed development in analyses. Once the females reached adulthood, I checked containers three times a week and recorded when each female died. This allowed me to measure female lifespan (from egg to death) and female development time of females that completed development.

I also conducted an experiment to assess the total lifetime fecundity of normal and distorter females. I produced separate jars containing approximately equal numbers of age-matched individuals for each female type. Immediately after these individuals completed development (i.e. before the female's cuticle reached full pigmentation), I isolated single females in a 35mm (in diameter) petri dish containing 0.5g of food. I placed two males into each of these containers (so females always had access to mating partners) and made 20 containers for each female type in total. Each week, I transferred the female (and males) into a new container with the same amount of food and counted the number of eggs she had laid in the past week. When males died, I would replace them with a new male from the colony so that females were always housed with 2 males. I

continued to do this until the female died. This allowed me to measure the number of eggs each female laid each week and the total lifetime fecundity for each female.

Facultative sex allocation in normal females

In order to assess whether normal females exhibit facultative sex allocation in response to environmental conditions I prepared a jar (125ml) containing a small amount of food and transferred approximately 200 late instar normal female nymphs and 200 males from laboratory stocks into the jar. I left females for 7 days so they had an opportunity to mature and mate and then transferred these females into petri dishes (35mm in diameter) containing 1.7g of food.

The experiment consisted of three treatments that differed in the number of females present in the petri dish. In the low-density treatment there were 2 females in each replicate dish, the medium-density treatment contained 10 females in each replicate dish and the high-density treatment contained 20 females in each replicate dish. I also kept 3 males in each dish with the females so that females always had access to mating partners. I produced 5 replicates for each treatment and transferred the females and males into new dishes weekly for 4 weeks. I terminated the experiment after 4 weeks since the data from the experiment addressing the fecundity of the female types suggested that females produce more offspring early in their reproductive period as opposed to later and time constraints precluded assessment over the entire female reproductive period. This experimental design allowed me to measure both the total sex ratio for each treatment and also how the sex ratio changed over time. If more than 20% of the females in a replicate died I stopped recording data from that replicate. This occurred for one replicate in the low-density treatment in week three and one replicate in the medium-density treatment in week four. I recorded the sex of the offspring that developed from each container to get a measurement of the sex ratio for each replicate each week.

Distorting element frequency in populations cages

I wanted to determine the frequency and stability of the distorting element in *L.* nr. *bostrychophila* populations. In order to do this, I conducted a laboratory experiment to

determine the stable frequency of the distorting element in mixed population cages and if the frequency of the distorting element changed over time depending on the initial population structure. I transferred 300 individuals into glass jars (125ml) containing 20g of food (1:7.5 mixture of Rice Krispies to cracked wheat). All treatments initially started with 100 males and 200 females, however, the ratio of distorter to normal females differed between treatments. There were two treatments, one that was started with an equal amount of normal and distorter females (i.e. 100 normal females and 100 distorter females) and the other started with 7 times the amount of normal females to distorter females (i.e. 175 normal females to 25 distorter females). The rationale for the treatment with fewer distorter to normal females is that in natural conditions it is more likely that distorter females would invade normal female populations (due to the fact that distorter females do not produce males and so cannot reproduce without living in close proximity to normal females). I also wanted to assess whether the different treatments would reach stable and similar distorter female frequencies over time.

I prepared eight replicates for each treatment and sampled population jars every 4 months for 12 months. Sampling consisted of isolating 100 randomly selected females from the population and extracting total DNA from 40 of these individuals using 20ul of PrepMan Ultra (Life technologies) to obtain 10ul of DNA. In order to determine whether an individual was a normal or distorter female, I made use of two sets of mitochondrial primers (that I designed to sequence the mitochondrial genome of each female type) that would amplify approximately 2000bp of either normal or distorter female DNA (Perlman et al. 2015). As mitochondria are in perfect linkage with the distorting element (since it is maternally transmitted with 100% efficiency), mitochondrial primers can reliably track the frequency of the distorting element in populations (Chapter 3). In addition, I conducted a preliminary assessment of the effectiveness of the primer sets for identifying the reproductive mode of a female in which I attempted to amplify DNA from specimens for which I knew the identity (i.e. the female type). I found that these primers amplified DNA only from the target female type so they were able to give accurate information about whether an individual was a normal or distorter female. I performed PCR for each individual with both of these primer sets (Supplementary Table 2-1 for primer

information and PCR conditions). If I was unable to amplify DNA using either of these primer sets I would run both PCRs again and if I was still unable to amplify DNA I assumed that the DNA extraction process was unsuccessful and selected a new individual to replace the negative sample. At the first sampling point (i.e. four months) I also froze two jars of each treatment to gain a better idea of the total number of individuals in the population jars and the relative number of males to females.

Data Analysis

I analyzed all data in RStudio v3.1.0. (R Core Team, 2014). The supplementary information includes summaries of the models used in analyses, including the AIC and parameter estimates (Supplementary Tables 2-2 to 2-7). I analyzed both female longevity data and development time data using Cox proportional hazards survival analyses with the survival package (Therneau and Granbsch 2000). I included female type as the explanatory variable and clustered observations by the container the females were raised in. I analyzed data measuring lifetime fecundity in two ways. I looked at whether the total amount of offspring produced (via egg counts) differed using a generalized linear model with female type as the explanatory variable and with a Poisson distribution. I found the data were overdispersed and therefore refit the model taking that into account Additionally, I examined whether the distribution of eggs produced by females changed over time. I did this to investigate whether normal females and distorter females had different egg laying patterns over time. I also analyzed this data using a generalized linear model with a Poisson distribution, including the week in the experiment and female type as explanatory variables (as well as a quadratic term since data were not linear) and including individual as a random factor. I applied a model selection process, choosing the model that minimized the AIC.

I analyzed data measuring facultative sex allocation in normal females using a generalized linear model with a binomial distribution. I used a model selection process, choosing the model that minimized the AIC and including female density and the week the data was collected as explanatory variables and the replicate as a random variable. I also analyzed data measuring the frequency of the distorting element in population cages

using a generalized linear model with a binomial distribution. In this analysis, I included the time the cages were sampled (time) and the initial frequency of distorter to normal females (treatment) as explanatory variables (as well as a quadratic term for the time the culture was sampled) and the replicate as a random variable and used a model selection procedure to select the model with the lowest AIC. For both the normal female sex allocation experiment and the population cage experiment I found the data were overdispersed and therefore refit the top models taking that into account. All models were assessed to determine whether the data fit the model assumptions (via residual analysis etc.) and were only used if appropriate.

Results

Fitness differences between female types

Longevity/development of normal and distorter females

Both development time and longevity differ between normal and distorter females. Distorter females take significantly longer than normal females to develop (Cox proportional hazard, $p=0.0014$) (Figure 2-2A), with distorter females taking on average 50.2±2.3 days to develop while normal females take 41.1±1.5 days to develop. Distorter females also do not live as long as normal females (cox proportional hazards, $p>0.0001$) (Figure 2-2B), with distorter females living on average 143.0±2.9 days and normal females living 168.6±10.2 days.

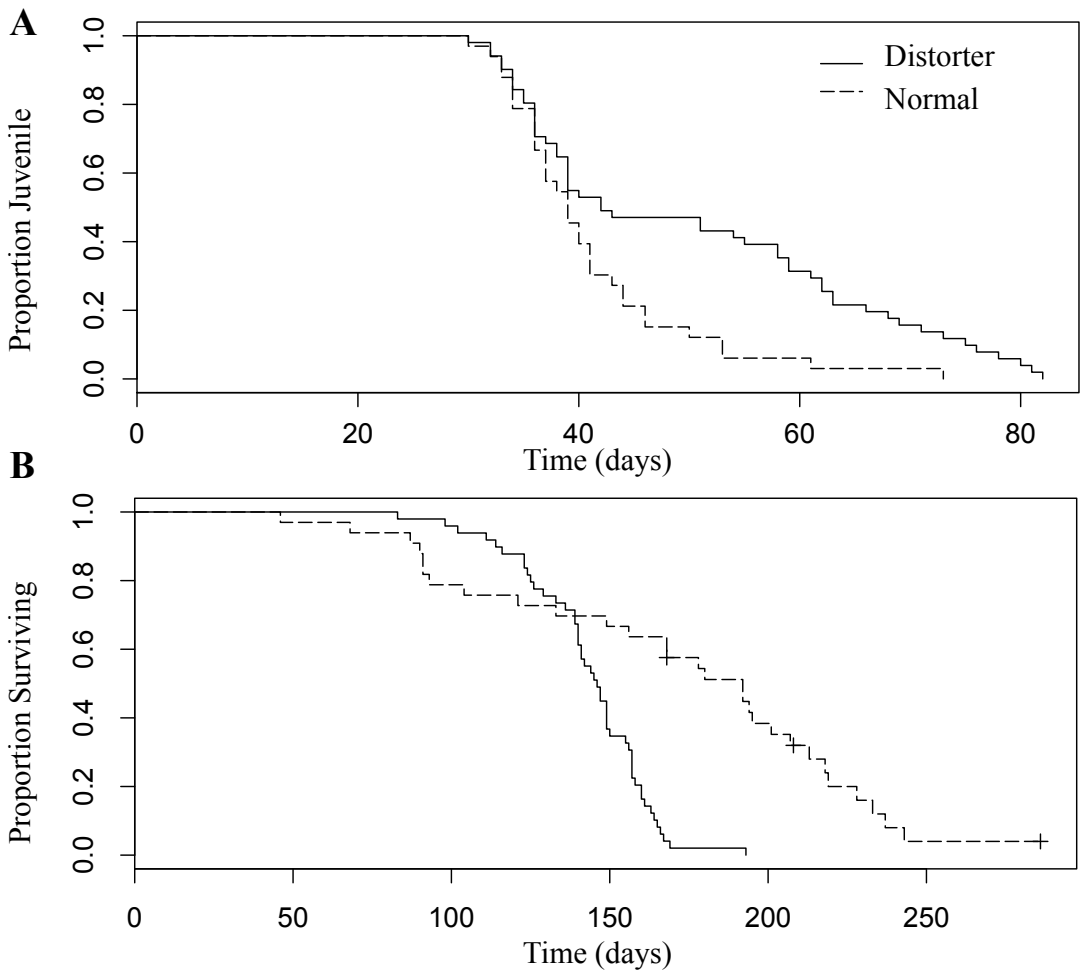

Figure 2-2. Normal (n=32) and distorter (n=51) female *L*. nr. *bostrychophila* development time and longevity. **A.** Female development time in days (from oviposition) for normal and distorter females, as a function of the proportion of individuals that are still juvenile. **B**. Normal and distorter female longevity in days (measured from oviposition). Censored data (individuals for which I could not assign a date of death) are marked with '+'.

Fecundity of normal and distorter females

Normal females lay significantly more eggs in their lifetime than distorter females (generalized linear model: $p>0.0001$) (Figure 2-3A). Additionally, normal females and distorter females have different egg laying patterns over time, with a significant interaction between the female type and the number of eggs a female lays in a week (generalized linear model: $p>0.0001$) (Figure 2-3B). The top model to describe the number of eggs a female lays over time included female type, the week the data was collected, and an interaction between the week the data was collected and female type.

Additionally, as the data was non-linear, a quadratic term (week) was included in the top model. Adding individual as a random effect did not improve the model and so was not included in the top model.

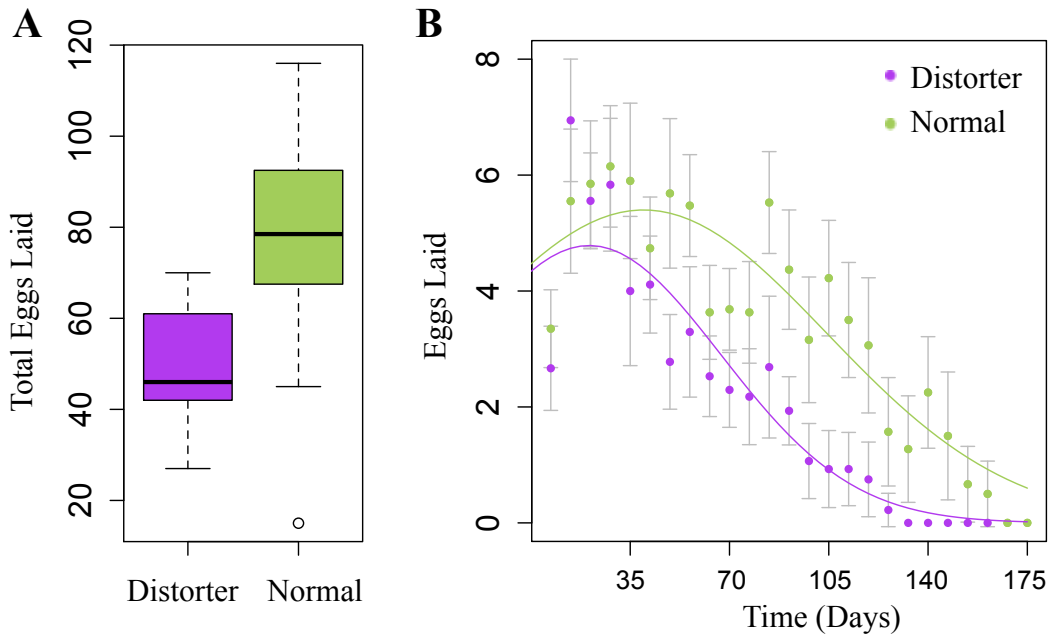

Figure 2-3. Fecundity differences between normal and distorter *L*. nr. *bostrychophila*. **A**. Total eggs laid by distorter (n=18) and normal (n=20) females over the entire experimental period. **B**. Number of eggs laid each week by distorter and normal females during the experimental period. Normal females lay more eggs than distorter females, with both female types laying similar amounts of eggs early in their reproductive period but distorter females laying less eggs than normal females as they age.

Facultative sex allocation in normal females

The top model to describe the sex ratio normal females produced included female density and the week the data was collected. The top model did not retain the replicate as a random factor. Normal females produce a female biased sex ratio over the entire experimental period (Sex ratio (%male)= 40.3±11.6, 32.2±8.1, 30.0±7.6 for low, medium and high density treatments respectively). Early in their reproductive period, females produce a slightly male biased sex ratio with more female offspring being produced over time (generalized linear model: $p<0.001$). Additionally, the sex ratio normal females produce is dependent on the density treatment, with a more female biased sex ratio being

produced in the medium and high density treatments compared to the low density treatments (generalized linear model: p=0.015) (Figure 2-4).

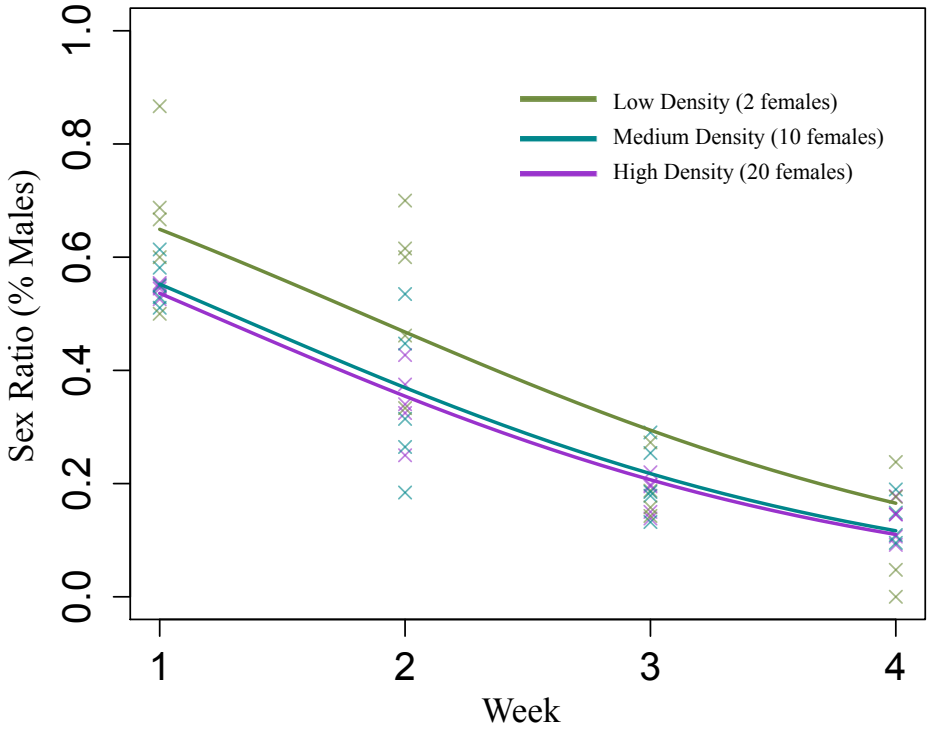

Figure 2-4. Sex ratio (proportion male) of offspring produced by normal females in response to female density. Females produce a more female biased sex ratio as they age. Females in the low-density treatment produced a more male biased sex ratio over the experimental period; however, there was no difference in the sex ratio produced by females in the medium and high-density treatments. Raw data points are plotted with lines indicating the sex ratio predicted for each treatment from the top model.

Frequency of the distorting element in population cages

The top model to describe the frequency of the distorting element in population cages included the treatment (i.e. the initial proportion of distorter to normal females), time, and an interaction between treatment and the time sampled. Additionally, as the frequency of distorter females in population cages varied non-linearly over time, time was also included as a quadratic term in the top model. The replicate was not retained in the top model as a random factor. In the treatment that initially contained a low frequency of distorter to normal females, the proportion of distorter females ranged from 10 to 55 % over the course of the experiment while in the treatment that initially contained an equal frequency of distorter to normal females the proportion of distorter females ranged from

50 to 95% (Figure 2-5). The two treatments did not reach the same frequency of distorter females over time. The frequency of distorter females in cages changed over time for population cages from both treatments. Additionally, the frequency of males decreased and the total density in the population cages increased for both treatments in the first four months of the experiment (Supplementary Table 2-8). Although there was an interaction between the time the population was sampled and the treatment suggesting that the treatments differed in their compositions over time, the overall trend in the different treatments was similar, with an initial rise in the frequency of distorter females in the population followed by a drop at the final time point sampled (12 months).

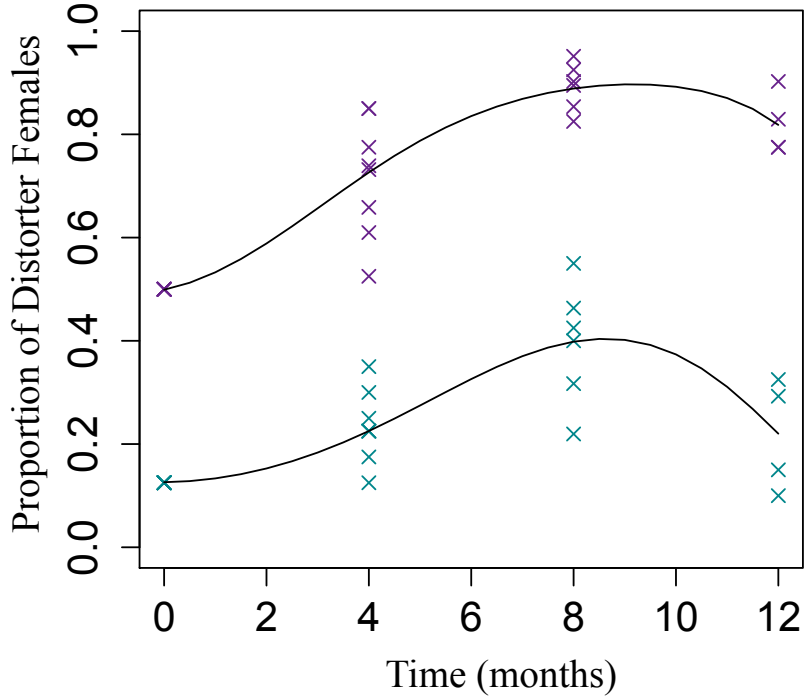

Figure 2-5. Proportion of distorter females (and the distorting element) in population cages started with either a 1:1 (purple) or 1:7 (blue) ratio of distorter to normal females. Lines indicate the predicted frequency of distorter females from the top model. Raw data points are plotted. The frequency of distorter females in populations initially increased in both treatments and decreased at the final time point sampled, however, the manner in which the populations changed was different depending on the initial frequency of distorter females in the culture (generalized linear model: $p>0.0001$).

Discussion

The impact selfish genetic elements that manipulate host reproduction have in shaping the ecology and evolutionary trajectory of their host is of growing interest to biologists. However, relatively few systems that contain these elements have been studied in any detail. *Liposcelis* nr. *bostrychophila* populations provide a new and unique opportunity to study a selfish genetic element that causes its host to produce exclusively female progeny, but still coexists in populations with hosts that do not carry the selfish genetic element and produce a mixed sex ratio. I investigated the ecology of *L.* nr. *bostrychophila* to determine both how the distorting element persists over time. I found that females that carry the distorting element have substantially reduced fitness compared to females that do not carry it. Specifically, females that carry the distorting element take longer to develop, do not live as long, and have a lower fecundity than females who do not carry the distorting element. Additionally, I have uncovered evidence for facultative sex allocation in females that do not carry the distorting element, with these females altering the sex ratio of their offspring in response to density and over their reproductive period. Finally, my exploration into the frequency of the distorting element in populations has revealed that populations appear robust to changes in the frequency of the distorting element, with populations persisting even at very high frequencies of the distorting element.

In systems that harbour selfish genetic elements resulting in female biased offspring production, fitness differences between individuals carrying the selfish genetic element and those not carrying it are important in population dynamics (Hatcher and Dunn 1995). I found that distorter females have substantially lower fitness than normal females, both in terms of longevity, fecundity, and development time. In this system it seems that the longevity differences between the female types likely drives the fecundity differences I observed. I explored this by examining whether distorter females and normal females produced different amounts of eggs over time. I found that at the beginning of their reproductive period, distorter and normal females produce similar amounts of eggs but that over time, distorter females start producing fewer eggs than normal females, likely because they are declining sooner than normal females. Therefore,

I suggest that the differences in longevity between normal and distorter females drive differences in fecundity between the two female types. The fecundity differences in turn likely contribute to the persistence of the distorter phenotype by balancing out, to some extent, the reproductive advantage distorter females have over normal females.

In this investigation I also found that normal females produce a female biased sex ratio and exhibit facultative sex allocation. However, both the mechanism of facultative sex allocation and reason for this behaviour is difficult to interpret. I found that females produce offspring with a slight male bias early in their reproductive period, but that over time females begin to produce an offspring sex ratio that becomes more female biased, producing approximately 80% female offspring later in their reproductive period. This pattern is similar to what was observed in mealybugs (*Planococcus citri*), where females were observed to produce a more male biased sex ratio early (and very late) in their reproductive period (Ross et al. 2010b, 2012). In this species as well, it is uncertain why this pattern exists; however, a possible reason is that males are produced earlier to ensure that females have mating partners. This would be adaptive if there is uncertainty that females will meet unrelated males early in their adult life (Ross et al. 2012). I also found that females that are housed with fewer females also produce a more male biased sex ratio. The reason for this is a mystery given that it is counter to what it observed in many systems (Hamilton 1967, Ross et al. 2010b). However, it should be noted that the ratio of males to females also differed between the density treatments in this study. This was a necessary consequences of ensuring that females in the treatments had mating partners over the experimental period, however, it does make the results of this experiment more difficult to interpret as the greater male bias produced by females in the low density treatment may have been a consequence of altering the ratio of males to females rather than the manipulation of female density. Therefore, it may be warranted to investigate facultative sex allocation in response to density in *L.* nr. *bostrychophila* further.

Over the entire experimental period, normal *L.* nr. *bostrychophila* females produced a female biased sex ratio (approximately 35% male). This reason that females produce this sex ratio is unclear and we likely need more information about the ecology

of this species in natural populations to resolve this mystery. For instance, it is possible that the reason normal females produce a female biased sex ratio in the laboratory is because they have plentiful resources and females have a greater reproductive value in these conditions. However, in recent field collections, the *Liposcelis* nr. *bostrychophila* individuals I collected (along with the other *Liposcelis* species) displayed extremely female biased populations suggesting that a female bias may be present in the field as well as the laboratory (Chapter 4).

Given that there was a strong female bias in laboratory settings and a female bias also found in natural populations, it is probable that normal females produce a somewhat female biased sex ratio in natural populations. Given this, it is interesting to consider how normal females producing a female biased sex ratio would affect the population dynamics of the entire system in natural settings. Theoretical studies suggest that in systems with selfish genetic elements causing female biased offspring production with 100% transmission efficiency (resulting in 100% female offspring), the sex ratio produced by uninfected individuals should not depend on the selfish genetic element due to restricted gene flow from infected to uninfected individuals (Werren 1987, Hatcher and Dunn 1995). Therefore, the sex ratio that normal females produce is not likely to be a result of the presence of the distorting element in this system. However, the fact that normal females will produce a female biased sex ratio, in addition to the fact that they will alter the sex ratio of their offspring in certain conditions, likely affects the persistence of the distorting element in this system.

In the population cage experiment examining the frequency of the distorting element in populations over time, I found that over the 12-month period that I collected data, populations that originally had different proportions of distorter to normal females did not reach the same proportion of distorter to normal females over the course of the experiment. Additionally, I found that although there was a significant interaction between when populations were sampled and the initial population structure, in all populations the proportion of distorter females initially increased followed by a decrease in the proportion of distorter females at the last time point measured. Since the distorting

element did not reach a stable frequency by the end of the sampling period, I was not able to conclusively determine whether the distorting element is able to persist over time. However, since the frequency of the distorting element fluctuated widely (from 10% to 95% in different population cages) without causing either extinction in any population cages or the loss of the distorting element from populations, populations appear to be fairly robust to changes in frequency of the distorting element over time. Additionally the fluctuations observed in the frequency of the distorting element over time (i.e. the initial increase in the distorting element followed by a decrease) may have been a result of frequency dependent population dynamics, as theoretical studies suggest this may be important in populations with selfish genetic elements (Werren 1987). I believe that the distorting element is able to persist in populations over time, and that density dependent processes may regulate the frequency of the distorting element over time. Specifically, I believe that the fitness of distorter females may decrease as the population density increases (since the population density increased dramatically in the first few months of the experiment) (Supplementary Table 2-8)(Werren 1987) leading to the decrease in the distorting element later in the experiment in population cages. Collecting data for a longer period, however, would allow me to determine more conclusively what the fate of the distorting element in *L.* nr. *bostrychophila* populations is.

I also found that the proportion of males in cages dropped dramatically at the first sampling point (i.e. in the first 4 months). Although this result seems intuitive given what we now believe about the female biased sex ratio normal females produce, it is interesting that these colonies are able to persist with such a low proportion of males. We know very little about male mating dynamics in this system and how males may play a role in the persistence of the distorting element. This would be an interesting area of future study since, given the low frequency of males in colonies; male dynamics may play a large role in the persistence of the distorting element. Male limitation has been linked to large ecological consequences such as a reversal of gender roles in the butterfly *Acraea encedon* (Jiggins et al. 2000). Male mating capacity has also been proposed to be an important driver of the persistence of selfish feminizing agents (Hatcher et al. 1999). Even if males are able to mate with and fertilize all females in a colony, there still may be

an impact on population dynamics if females have to wait some time after they are reproductively mature to mate. This might be particularly important in this system given that distorter females do not live as long as normal females so any delay in mating may negatively affect them disproportionately more than normal females. Investigating the role of male mating behaviour in the persistence of the distorting element in *L.* nr. *bostrychophila*, especially given that very little is known about the ecology of males in this system, may be an important area of future research.

A remaining question regarding the persistence of the distorting element is why this element, and distorter females, are able to persist in populations at all, given distorter females' lower fitness than normal females and the female biased sex ratios normal females produce. It is suggested that if females carrying a selfish genetic element that causes a female bias have a much lower fitness than other females in the population, that the element should be purged from the population over time (Werren 1987). Given this, why are distorter females able to persist in laboratory populations (and the population cage experiment) and why are we able to find distorter females in the wild over time (i.e. in both 2010 and 2014 field collections) (Chapter 4)? Clearly, the population dynamics in this system are complex. More information about the population structure and dynamics of *L.* nr. *bostrychophila* in natural setting would be especially useful for answering these questions. However, from these experiments I believe that density depending dynamics and male mating dynamics may be important players in this system. The population cage experiment suggests that the relative fitness of distorter and normal females may depend on the density in colonies (since the frequency of distorter females decreased after population density increased) and that males are at a low frequency in populations and therefore may play a large role in population dynamic. Therefore, investigating these two factors in more detail (especially in more natural situations) may produce important information about how the distorting element persists over time.

Conclusions

Liposcelis nr. *bostrychophila* populations provide an exciting new opportunity to investigate how ecological and life history factors influence the persistence of a selfish

sex ratio distorting element in its host. Although the experiments in this study were conducted in laboratory settings and so may not reflect with complete accuracy conditions in the wild, I believe that we have uncovered some factors that affect the persistence of the distorter female phenotype in *L.* nr. *bostrychophila* populations. I found that fitness differences between females carrying the selfish sex ratio distorting element and those not carrying it and that sex allocation in females not carrying the distorting element likely contribute to the persistence of the distorting element in this system. Additionally, I found that populations are robust to changes in the frequency of the distorting element, with extreme frequencies of this element resulting in neither population collapse nor the disappearance of the distorter phenotype from populations. Interestingly, this system appears to be different in several ways from similar systems that have been investigated. Future investigation into the population dynamics in this system, especially information from wild populations, may provide insight into how genetic and ecological factors interact to allow the invasion a selfish sex ratio distorter into this system and generally, how selfish elements affect the evolutionary trajectory of a species.

Supplementary Information for Chapter 2

Supplementary Table 2-1. PCR primer sequence and thermocycling conditions used in the population cage experiment to determine whether individuals were distorter or normal females. Primer sets amplify an approximately 2000bp region of either distorter female (CO1) or normal female (ND5) mitochondria. Preliminary assessment confirmed that these primer sets are specific for the mitochondrial region of the female type they were intended to amplify.

Female type	Mitochondrial region	Primer name	Sequence	PCR conditions
Distorter	CO1	FCO1F1	TAATGCCCAAGTC CGGATGG	93°C×3min, (93°C×20sec,57°C×30sec, 68°C×3min)×40, 68°C×5min
		FCO1R1	TGCTCACACAATG AACCCCA	
Normal	ND5	MND5F1	CAATGAAGGTGG TATCCCCATA	93°C×3min, (93°C×20sec,57°C×30sec, 68°C×3min)×40, 68°C×5min
		MND5R1	GTCACCTTTTCTG GCGACTC	

Supplementary Table 2-2. Parameter values for the survival model assessing the effect of female type on female development time. A Cox proportional hazards model was used to analyze data and observations were clustered by the container females were housed in. The p value displayed is taken from the Wald test. N=84, SE=standard error, exp=exponent.

Parameter	coefficient	exp(coef)	SE(coef)	robust SE	z	P(>|z|)
Female Type (NF)	0.704	2.0219	0.2425	0.221	3.186	0.00144

Supplementary Table 2-3. Parameter values for the survival model assessing the effect of female type on female longevity. A Cox proportional hazards model was used to analyze data and observations were clustered by the container females were housed in. The p value displayed is taken from the Wald test. Four observations were censored due to uncertainty in the time of death of individuals. N=82, SE=standard error, exp=exponent.

Parameter	coefficient	exp(coef)	SE(coef)	robust SE	z	P(>|z|)
Female Type (NF)	-1.4513	0.2343	0.3126	0.3262	-4.448	8.65E-06

Supplementary Table 2-4. Parameter estimates and significance level in the generalized linear model assessing the total lifetime fecundity of normal and distorter *L.* nr. *bostrychophila.* Degrees of freedom=37, AIC=433.98.

Parameter	Estimate	SE	t value	P(>|t|)
Intercept	3.883	0.076	51.415	< 2e-16
Type (Normal)	0.468	0.094	4.959	< 0.001

Supplementary Table 2-5. Parameter estimates and significance level in the generalized linear model assessing the total fecundity of normal and distorter *L.* nr. *bostrychophila* each week over their life. Degrees of freedom=677, AIC=2808.

Parameter	Estimate	SE	z value	P(>|z|)
Intercept	1.467	0.064	23.078	< 2e-16
Week	0.067	0.015	4.464	<0.001
Type (Normal)	0.029	0.058	0.501	0.616
Week^2	-0.011	0.001	-11.071	< 2e-16
Type (Normal):Week^2	0.005	0.001	9.107	< 2e-16

Supplementary Table 2-6. Parameter estimates and significance level of parameters in the generalized linear model assessing the sex allocation of normal *L.* nr. *bostrychophila* over time and at different female densities. Degrees of freedom=56, AIC=284.14.

Parameter	Estimate	SE	t value	P(>|t|)
Intercept	1.361	0.178	7.658	<0.001
Density (Medium)	-0.405	0.169	-2.400	0.020
Density (High)	-0.471	0.158	-2.975	0.004
Week	-0.745	0.043	-17.417	< 2e-16

Supplementary Table 2-7. Parameter estimates and significance level of parameters in the generalized linear model assessing the frequency of distorter *L.* nr. *bostrychophila* in population cages over time. Treatment refers to the frequency of distorter to normal females the population cage was started with (either 1:1 or 1:7). Degrees of freedom=51, AIC=285.63.

Parameter	Estimate	SE	t value	P(>|t|)
Intercept	-0.004	0.053	-0.079	0.937
Treatment(1:7)	-1.932	0.093	-20.667	< 2e-16
Time	0.080	0.089	0.893	0.377
Time^2	0.060	0.022	2.679	0.010
Treatment(1:7):Time	-0.070	0.022	-3.233	0.002
Time:Time^2	-0.005	0.001	-3.610	0.001

Supplementary Table 2-8. Proportion of males and total population density in population cage experiment initially and at four month sampling period. The frequency of males decreased and the total population density increased over the first four months of the experiment in both treatments.

Treatment (distorter: normal females)	Replicate	Initial % males	% Males at four months	Initial density	Density at 4 months
1:7	1	0.333	0.266	300	7158
	2	0.333	0.290	300	6224
1:1	1	0.333	0.087	300	9429
	2	0.333	0.076	300	7349

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Chapter 3 - Extreme intraspecific mitochondrial polymorphism in a booklouse (Psocodea: *Liposcelis*): consequences of cotransmission with a selfish genetic element

Abstract

Genomic conflict affects not only the genomic elements that are in conflict with one another, but other parts of the genome as well. The effect of genomic conflict is especially pronounced when considering selfish genetic elements. Selfish genetic elements often cause substantial genomic changes in their host population over time. In *Liposcelis* nr. *bostrychophila*, a maternally transmitted selfish genetic element causes individuals that carry it to produce exclusively female offspring. Since this element is maternally transmitted with 100% efficiency, it affects the evolution of all other maternally transmitted elements (as they are cotransmitted). I investigated how cotransmission with this selfish genetic element affects the evolution of mitochondria in *L.* nr. *bostrychophila*. I found that females that carry the selfish genetic element (distorter females) and females that do not carry it (normal females) have extremely different mitochondria, with normal females having seven mitochondrial minichromosomes and distorter females having five, and the mitochondrial genes arranged in a different order in the different female types. Additionally, the nucleotide sequence in distorter and normal females are very different, with on average approximately 25% nucleotide sequence divergence in coding regions. The impact of these differences on mitochondrial function in normal and distorter females is uncertain; however, distorter females have a reduced longevity compared to normal females. Since mitochondrial function plays a role in aging, distorter females may not live as long as normal females due to impaired mitochondrial function.

Introduction

Selfish genetic elements (and genomic conflict in general) have a large impact on the evolution of their host's genome. This can occur as a result of both the genomic conflict itself and the spread of the selfish genetic element through the host population. For instance, a selfish genetic element is locked in antagonistic interactions between

genomic elements it is in conflict with. This conflict places a large selection pressure on genomic elements that suffer as a result of the selfish genetic element to evolve methods to resist the action of the selfish genetic element. For instance, the stalk eyed fly species *Cyrtodiopsus dalmanni* and *C. whitei* harbour a selfish X chromosome, which causes males carrying it to produce predominantly female offspring (Presgraves et al. 1997). The selfish X chromosome drastically reduces the fitness of the Y chromosome in males carrying the selfish X chromosome. This places a strong selection pressure on genes on the Y chromosome to resist the sex ratio distortion induced by the selfish X chromosome. This has led to the emergence of a suppressor on the Y chromosome, which counteracts the effect of the selfish X chromosome, restoring a more equal sex ratio in males carrying the selfish X chromosome (Presgraves et al. 1997). Suppressors on the Y chromosome have evolved in response to selfish X chromosomes in several lineages of flies, including stalk eyed flies and *Drosophila* species (Jaenike 2001).

Although selfish genetic elements by definition negatively affect other genomic elements, they can also increase the transmission of some elements in the genome. For instance, selfish genetic elements that are maternally transmitted are in linkage disequilibrium with other maternally transmitted elements (i.e. endosymbiotic bacteria, mitochondria, and female limited W chromosomes). This means, for instance, that an endosymbiotic bacterium that manipulates reproduction in its host and as a consequence rises to high frequency in its host population, will bring along with it the mitochondrial haplotype (or W chromosome in species with ZW sex determination) that it was originally associated with. Several studies have investigated the spread of mitochondrial haplotypes as a result of cotransmission with maternally transmitted endosymbiotic bacteria (or other cytoplasmic entities) that manipulate reproduction in their host. Generally, population mitochondrial diversity is reduced as a result of selfish endosymbionts since diverse mitochondrial haplotypes are replaced by the one associated with the reproductive manipulator (Ballard et al. 1996, Jiggins 2003, Shoemaker et al. 2004). However, there are also rare cases in which cotransmission with selfish genetic elements allows for the persistence of rare mitochondrial haplotypes and therefore result in greater mitochondrial polymorphism (Dyer et al. 2011).

Since mitochondria are the primary energy producers in cells, mitochondrial function is an important component of organismal fitness (Strohm and Daniels 2003). An individual's mitochondrial genotype has been linked to mitochondrial function and organismal fitness in several ways (Pichaud et al. 2012, Meiklejohn et al. 2013). For instance, Melvin & Ballard (2006) investigated the fitness effects of naturally occurring mitochondrial haplotypes in *Drosophila simulans.* They found that different mitochondrial haplotypes had different electron transport chain complex efficiencies measured through both the ADP:O ratio (a measure of overall electron transport chain efficiency) and H_2O_2 production (a measure of oxidative damage). Additionally, mitochondrial efficiency was related to individual longevity, with the mitochondrial haplotype with the greatest efficiency (i.e. highest ADP:O ratio and lowest H_2O_2 accumulation) associated with the longest-lived individuals. Given that mitochondrial function is important in organismal fitness, changes in mitochondrial diversity in a population, especially if it is driven by processes that do not involve selection on mitochondria such as cotransmission with other maternally inherited genetic elements, may have important population consequences. For example, the *Drosophila simulans* population mentioned above carries the endosymbiotic bacterium, *Wolbachia.* This endosymbiont is thought to contribute to the divergence of the mitochondrial haplotypes in this lineage (James and Ballard 2003), which in turn affects mitochondrial function (Melvin and Ballard 2006).

Over the course of mitochondrial evolution in animals, a major trend has been the transfer of mitochondrial genes into the nuclear genome. The products of these genes, along with genes of nuclear origin that have been co-opted to function in mitochondria, contribute to mitochondrial function and serve alongside mitochondrial genes to form the complexes of the ATP producing oxidative phosphorylation (OXPHOS) pathway, which is responsible for most of the energy production in cells (Rand et al. 2004). Therefore, complementarity between mitochondrial encoded and nuclear encoded subunits of the OXPHOS pathway is important for proper cell function, resulting in coevolution between nuclear and mitochondrial encoded parts of the OXPHOS pathway (Barreto and Burton

2013). Incompatibility between mitochondrial genes and nuclear genes whose products function in the mitochondria (cytonuclear incompatibility) can have severe consequences on organismal fitness and has even been proposed as an important player in speciation in some lineages (Ellison and Burton 2006, Ellison et al. 2008). Cytonuclear incompatibility may be particularly important to investigate in systems where a selfish genetic element is causing a mitochondrial haplotype to sweep through a population, as the mitochondrial haplotype may not be compatible with the nuclear background of all members of the population, especially if the mitochondrial haplotype is spreading over large geographic areas where it may not be well adapted to the nuclear background individuals carry.

Generally, mitochondria are well conserved in animals, with few changes from the ancestral configuration and organization over time (Boore 1999, Cameron 2014). Several animal lineages, however, have elevated rates of mitochondrial evolution (Oliveira et al. 2008). In addition, elevated mitochondrial evolution rates are associated with cytonuclear incompatibility and mitochondrial dysfunction in some lineages. For instance, the copepod *Tigriopus californicus* exhibits extreme intraspecific mitochondrial divergence, which is linked to cytonuclear incompatibility in crosses between allopatric populations (Ellison and Burton 2006). One of the animal lineages with rapidly evolving mitochondria, and also arguably the animal lineage with the oddest mitochondria, is the insect order Psocodea. Psocodea (formerly the orders Phthiraptera and Psocoptera) contains parasitic lice, booklice, and barklice, and is the only order in the animal kingdom to contain species with multiple mitochondrial chromosomes within the mitochondrial genome (Cameron 2014). There is a wide variety in the mitochondrial configurations of members of this lineage, with some species having only one mitochondrial chromosome (Li et al. 2013, Chen et al. 2014a), several having their mitochondrial genes arranged on two mitochondrial chromosomes (Wei et al. 2012, Chen et al. 2014b), and a few (including the head and body louse *Pediculus humanus,* whose mitochondrial genome contains 18-20 minichromosomes) having more than two mitochondrial chromosomes (Shao et al. 2009). Several species within the group Psocodea also harbour endosymbiotic bacteria and other elements that may act as selfish genetic elements (Perotti et al. 2004, Behar et al. 2010). For instance, human head lice

often have female biased population sex ratios and also can carry the endosymbiotic bacteria *Wolbachia* (Perotti et al. 2004). However, the effect of carrying selfish elements on the mitochondrial genome or genetic diversity in this group has never been investigated.

A recently discovered psocodean harbours a selfish genetic element (Perlman et al. 2015). The booklouse, *Lipsocelis* nr. *bostrychophila,* is a sexual species in which there is two distinct female reproductive phenotypes. One female type carries the selfish genetic element (designated the distorting element) and produces exclusively female offspring that carry the element as well (designated distorter females), while the other female type does not carry the distorting element and produces a mixed sex ratio (designated normal females). The distorting element is maternally transmitted, and is thought to be located in the nuclear genome of distorting females due to the fact that part of the nuclear genome found in distorter females are not found in other individuals in the population (Perlman et al. 2015). However, since mitochondria are maternally transmitted it is possible that the distorting element is a mitochondrial element. In any case, due to the similar transmission strategies of the distorting element and mitochondria, the presence of the distorting element in this lineage likely has affected the evolution of *L.* nr. *bostrychophila* mitochondria due to the linkage between these elements. In order to assess this, I investigated the mitochondrial genomes of normal and distorter females both to determine how the distorting element has affected mitochondrial evolution in this species and to more conclusively establish whether the distorting element is a mitochondrial element.

I sequenced the mitochondrial genome of normal and distorter females and compared the mitochondrial configuration as well as the similarity of mitochondrial coding regions in normal and distorter females to determine how the distorting element is affecting mitochondrial evolution in *L.* nr. *bostrychophila.* I expected that the presence of the distorting element in this system as well as the elevated mitochondrial evolution in this lineage would affect mitochondrial diversity, with distorter females having a different mitochondrial haplotype from normal females. I also expected that, like the

majority of other *Liposcelis* species that have had their mitochondrial genomes sequenced, both female types would have their mitochondrial genes arranged on two mitochondrial minichromosomes. The results of this investigation have now been published in Perlman et al. (2015). In addition to this, I compared the mitochondria of *L.* nr. *bostrychophila* to other *Liposcelis* species that have had their mitochondrial genomes sequenced. This is the first study that has investigated the effects of a selfish genetic element that manipulates reproduction on mitochondrial evolution in a species with rapidly evolving mitochondria.

Methods

Colony Information

Liposcelis nr. *bostrychophila* was originally collected from the Chiricahua Mountains, Arizona (approx. 31.9N,109.3W), in 2010. The colonies were maintained at 27°C and approximately 75% humidity using a saturated NaCl solution. I kept colonies in glass jars (125ml) filled with a 1:10 mixture of Rice Krispies (Kellogg's) to cracked wheat (Bob's Red Mill). I kept normal and distorter *L.* nr. *bostrychophila* females in separate cultures, transferring males into the distorter female colonies weekly (so distorter females had mating partners).

Sequencing and annotation of L. nr. *bostrychophila mitochondrial genomes*

More information about the sequencing and annotation of normal and distorter female mitochondrial genomes can be found in Perlman et al. (2015). The sequencing of normal and distorter *L.* nr. *bostrychophila* mitochondrial genomes was a collaborative effort with Finn Hamilton. Finn was involved in the Illumina sequencing for both female types, preparing and assembling the Illumina sequence assemblies for each female type.

We used a combination of Illumina and Sanger sequencing to sequence the mitochondrial genome of normal and distorter *L.* nr. *bostrychophila*. For the Illumina sequencing, we extracted DNA from 35 individuals of each female type using a DNeasy Blood and tissue kit (Qiagen) according to manufacturer directions. Beckman Coulter Genomics constructed and sequenced libraries for each female type (~4*10^7 100-bp PE

reads per line). We generated draft assemblies for each female type with Ray v2.20 (k=31). We also searched the assemblies for mitochondrial genes with tblastx, using the sequenced mitochondrial genome of *L. bostrychophila* as the query and used the retrieved gene regions (400-800bp) as seeds in mitoBim (proofreading mode) to validate assemblies.

We expected to obtain two linear assemblies for each female type. This is because we expected each female type to have two mitochondrial minichromosomes (since their close relative *L. bostrychophila* has two mitochondrial minichromosomes), and using this method we would obtain one linear assembly corresponding to each minichromosome. However, contrary to our expectation we obtained five or more distinct assemblies for each female type. Therefore, in order to resolve whether the mitochondrial genomes were assembled correctly and to sequence the remainder of the circular chromosomes, I designed primers near both ends of each assembly in both directions (one set to confirm the assembly and the other to sequence the remainder of the circular chromosome). I performed PCR with these primers (Supplementary Table 3-1 for PCR conditions and primer sequences used) and Sanger sequenced the products. In some cases I cloned the PCR products using a StrataClone PCR cloning kit (Agilent Technologies) following the manufacturers directions before sequencing. All Sanger sequencing was carried out with DNA extractions from 16 individuals in 60µl of PrepMan Ultra (Life Technologies) to obtain 30µl of DNA.

I annotated the protein coding regions and rRNA regions in the mitochondrial genomes of each female type. For the protein coding regions, I extracted the open reading frames longer than 120bp from the completed mitochondrial assemblies using getorf (EMBOSS). I annotated these regions using blastp searches with the non-redundant protein database (National Center for Biotechnology Information). This investigation also allowed me to explore whether any ORF in the distorter female mitochondrial genome appeared likely to be the cause of the distorter phenotype. I manually annotated rRNA regions by aligning the 12S and 16S regions in *L. bostrychophila* to our assemblies in

Geneious v6.1. Mitochondrial genome sequences have been deposited in GenBank under accessions KP641133, KP657691–KP657699, and KP671844–KP671845.

Additionally, I wanted to explore the possibility of radically different mitochondrial haplotypes in different members of the same female type. In order to investigate this, I assessed whether there were structural differences between the mitochondria of different individuals of the same female type. I examined five regions of the distorter and normal female mitochondria with an expected size range from 1,200bp to 3,000bp (Supplementary Table 1 for PCR conditions and primer information). Using DNA extractions from single females in 20µl of PrepMan Ultra (to yield 10µl of DNA) I amplified each of the five regions in eight individuals of each female type. Since females of the same female type are expected to have a similar mitochondrial conformation, I would expect that I would obtain the same size PCR product when comparing the same mitochondrial region in different females of the same female type. I therefore compared the PCR band sizes to the expected band size (from the mitochondrial assembly for that region) to determine both whether the primers were able to amplify a product and whether the product was of the expected size in all individuals of the same female type.

Mitochondrial evolution in Liposcelis

I obtained the nucleotide sequences for protein coding regions of the *Liposcelis* species that have sequenced mitochondrial genomes in GenBank (NCBI). These species are *L. decolor*, *L. entomophila*, *L.paeta*, and *L. bostrychophila* (accessions NC_023839.1, NC_025503.1, NC_025504.1, NC_025505.1, NC_25506.1, JN645275.1, JN645276.1). I also included the protein coding sequences from *Campanulotes bidentatus*, the small pigeon louse, in the analysis as the outgroup (accession NC_007884). I aligned the nucleotide sequence of protein coding regions of these species, along with the distorter and normal female mitochondrial haplotypes of *L*. nr. *bostrychophila*, in Geneious v6.1 using a MUSCLE translation alignment with a gap penalty of -2 and 10 iterations. I excluded the ATP8, ND2, ND4L, and ND6 genes from further analyses since these regions are too short and variable to confidently align. The alignments of the remaining protein coding regions (ATP6, CO1, CO2, CO3, COB, ND1, ND3, ND4, and ND5) were

concatenated and used to assemble a phylogeny using the PhyML plugin in Genious v6.1 (Guindon & Gascuel, 2003). I assembled the phylogeny with the HKY85 model, 1000 bootstrap replicates, and an SPR topology search.

Results

The mitochondria of normal and distorter *L.* nr. *bostrychophila* females are extremely different from each other: having both a different number of mitochondrial minichromosomes (five in distorter females and seven in normal females) and a different configuration of the mitochondrial genes within these chromosomes (Figure 3-1). Not only are the mitochondria radically different in terms of configuration, but the protein coding and rRNA regions are also extremely divergent between the females types, with an average of 73.1% similarity in these regions (Table 3-1).

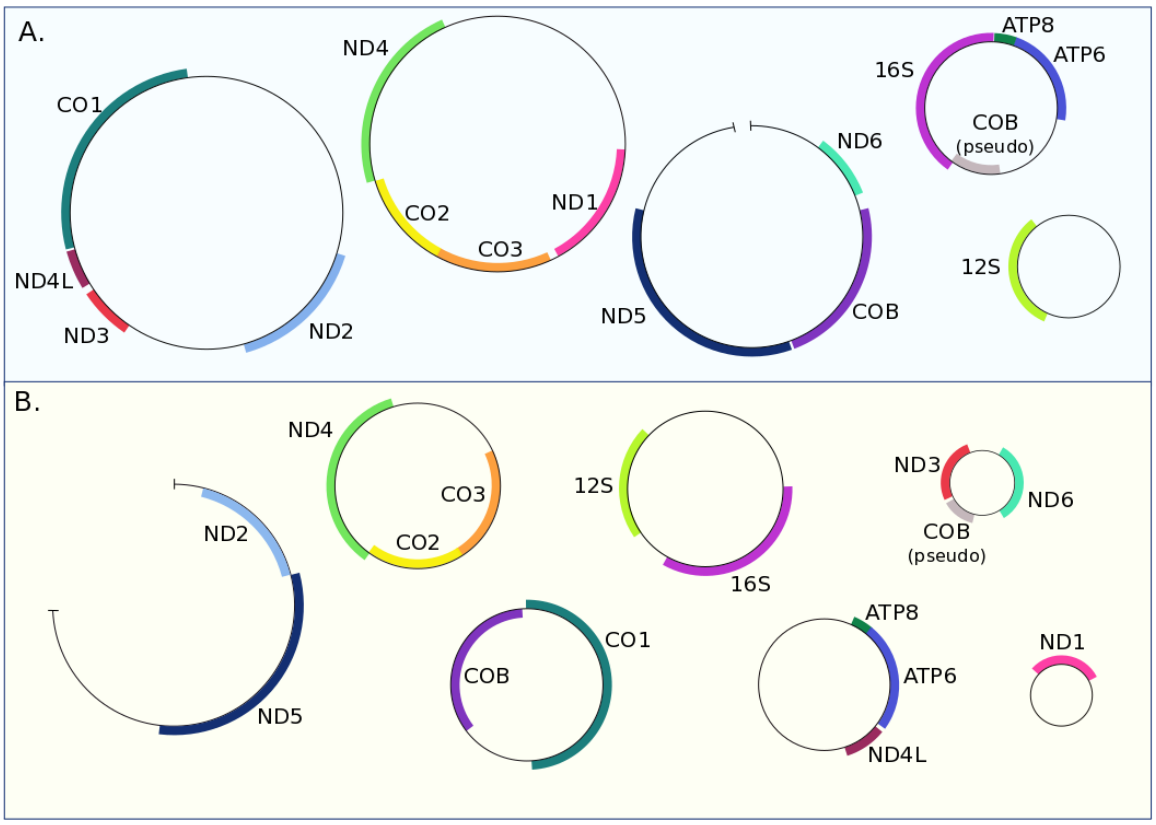

Figure 3-1. Mitochondrial configuration of **A**. *Liposcelis* nr. *bostrychophila* (Distorter), and **B.** *Liposcelis* nr. *bostrychophila* (Normal). Protein coding regions and rRNA regions are identified. Genes on the outside of the circle are in the forward direction while those on the inside are in the reverse direction. Circles are labeled for the largest gene in the circle and the sizes are as follows: Distorter female minichromosomes: CO1 (5,626bp), ND4 (5,312bp), ND5 (~4,600bp), 16S (2,746bp), 12S (2131bp). Normal female minichromosomes ND5 (>3,700bp), ND4 (3,426bp), CO1 (3,147bp), 16S (2,746bp), ATP6 (2,714bp), ND6 (1,354bp), ND1 (1,275bp). Genbank accessions: KP641133, KP657691-KP657699, KP671844-KP671845 (Perlman et al., 2015).

Table 3-1. Percent nucleotide similarity in coding regions between normal and distorter *Liposcelis* nr. *bostrychophila* females. MUSCLE alignments (translation alignments for protein coding genes and standard alignments for rRNA regions) were used to compare gene regions.

Gene	% Nucleotide Similarity
ATP6	75.4
ATP8	65.4
CO1	76.6
CO2	73.9
CO3	70.6
COB	76.8
ND1	76.1
ND2	73.8
ND3	76.8
ND4	72.4
ND4L	75.9
ND5	70.9
ND6	53.1
12S	80.1
16S	80.1

I was unable to sequence a small portion of the mitochondrial genome for each female type. Although I did not obtain the sequence for these regions, I was able to estimate the approximate size of these minichromosomes using the band size from the PCR reactions. Additionally, in the distorter female mitochondrial genome, the region identified as ND6 using blast searches contained a stop codon at position 44 in the coding region. I re-sequenced this region to ensure the sequence was correct and also performed blast searches using ND6 from *L. bostrychophila* as a query and the Illumina sequence assembly as a database and did not find errors in the sequence or any other regions with significant similarity to ND6. Therefore, to the best of my knowledge, the sequence within this region is correct and is the only region in the distorter female genome with significant similarity to ND6 in its close relative *L. bostrychophila.*

For each of the eight normal and distorter females that I assessed mitochondrial polymorphism within female types, I was able to amplify a band of the expected size with the same primer sets used to sequence the mitochondria of each female type. The primers used generally spanned more than one gene, indicating that the gene order in females of the same female type is likely the same. Additionally, this indicates that there is little mitochondrial variation within female types, as given the rapidly evolving nature of mitochondria in this lineage, I would otherwise expect to not amplify the same size bands for all individuals of the same female type.

The relationship between *Liposcelis* sp. based on the mitochondrial protein coding genes is shown in Figure 3-2. As expected, distorter and normal female mitochondrial genomes are most closely related to each other; however, the level of divergence between the two female types is large.

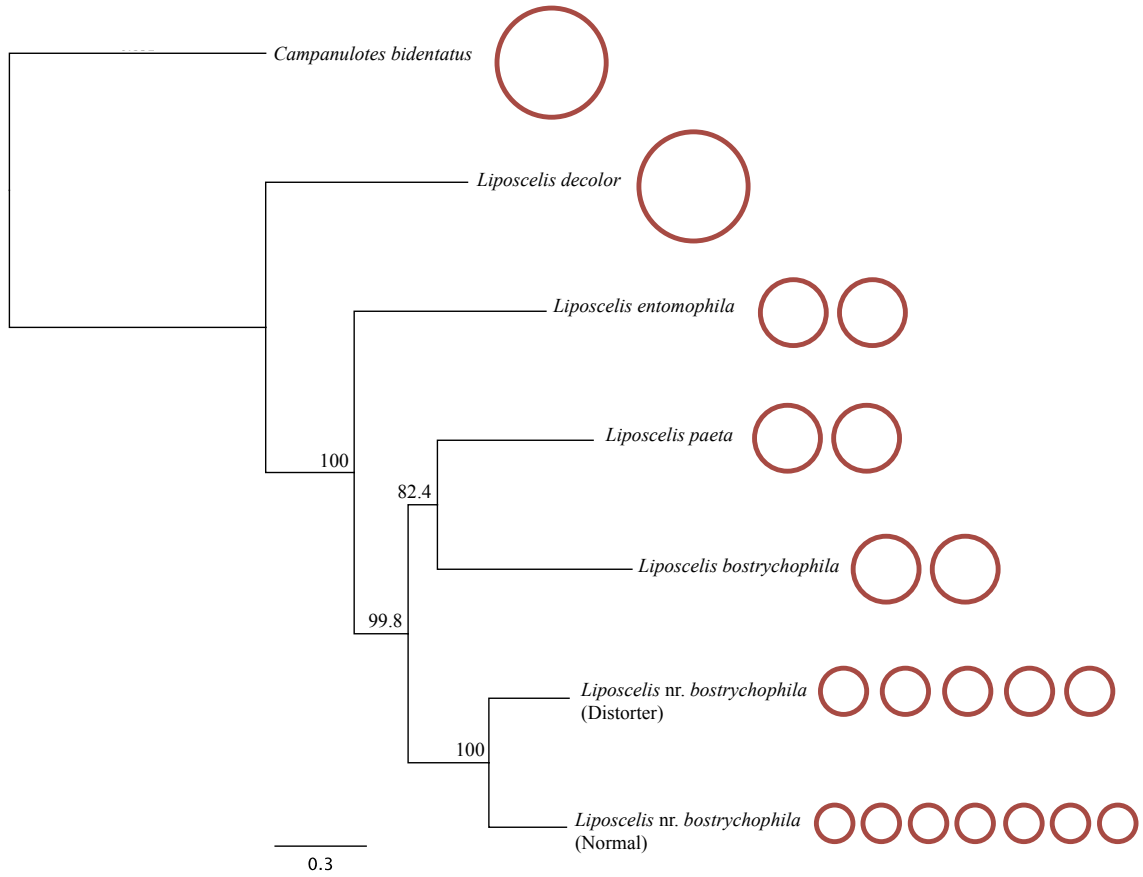

Figure 3-2. Phylogeny of *Liposcelis* species using a MUSCLE translation alignment of protein coding regions (ATP6, CO1, CO2, CO3, COB, ND1, ND3, ND4 and ND5). Bootstrap support for branches are shown at nodes. *C. bidentatus* (Psocodea: Amblycera) was included as an outgroup. The circles beside the species name indicates the number of mitochondrial chromosomes that species has.

Discussion

I sequenced the mitochondrial genomes (from a pooled sample of 35 individuals for each female type) of both normal and distorter *L.* nr. *bostrychophila* and found that they are radically different from each other. The mitochondrial genome of normal and distorter females have an extremely different configuration from each other; with normal females having their mitochondrial genes arranged on seven minichromosomes and distorter females having their mitochondrial genes arranged on five minichromosomes. Additionally, the mitochondrial gene order in normal and distorter females is almost entirely different, with only the ATP6/ATP8 and ND4/CO2/CO3 genes in the same order in the two female types. This is the first documented case of different members of the

same species in an animal having different numbers of mitochondrial minichromosomes as well as such a drastically different mitochondrial gene order. Additionally, this is also the first *Liposcelis* species sequenced to have more than two mitochondrial minichromosomes. Not only do distorter and normal females have a different mitochondrial configuration, but also the nucleotide sequence in the coding regions is very dissimilar, with on average approximately 25% divergence in coding regions. This level of sequence divergence in members of the same species is almost unprecedented. The copepod, *Tigriopus californicus*, has been reported to have greater than 20% divergence in mitochondrial genes (Burton et al. 2007). However, the *T. californicus* individuals sequenced were from different populations and were not expected to be interbreeding, unlike normal and distorter *L.* nr. *bostrychophila*, that must interbreed in order for distorter females to persist.

As this is the first record of intraspecific mitochondrial divergence of this magnitude in interbreeding members of a population it raises questions about the evolution and functionality of the mitochondria in this lineage. For instance, how does this extreme diversity in mitochondria affect cytonuclear evolution and are there cytonuclear incompatibilities in this system? Since normal females give birth to all of the males in the population, males have the normal female mitochondrial haplotype and the corresponding nuclear background. Distorter females, however, must mate with males to reproduce. Therefore each generation, distorter females are inheriting half their autosomal genes from males whose cytonuclear genes have evolved to function well with normal female mitochondria. Given the level of divergence between normal and distorter female mitochondria, I would expect that distorter females might experience cytonuclear incompatibility.

There are two pieces of evidence that support this hypothesis. First, a recent study found that the mitochondria have a different appearance in the rectal glands of normal and distorter females (Perlman et al. 2015). In normal females the mitochondria appear normal, but in distorter females the mitochondria appear abnormal, without extensive cristae and with few intact scaliform junctions. The significance of this observation in

terms of rectal gland function and female fitness is unknown, however I would speculate that the unusual appearance of the mitochondria in the rectal glands reflects mitochondrial performance in this tissue. For instance, reduced efficiency of the electron transport chain in this tissue may result in either the loss of mitochondria membrane potential or the generation of reactive oxygen species (such as superoxide or H_20_2), which may damage mitochondria in these tissues. Additionally, distorter females do not live as long as normal females (Chapter 2). Given that mitochondrial function (both the efficiency of the electron transport chain and the generation of reactive oxygen species) is implicated in aging (Melvin and Ballard 2006), and that cytonuclear incompatibility has been linked to impaired mitochondrial function (Ellison and Burton 2006), I believe that distorter female mitochondria may not function well in the normal female nuclear background resulting in reduced longevity compared to normal females.

I found that despite the level of gene rearrangement and unusual mitochondrial genome configuration in normal and distorter females, both female types still contain the rRNA and protein coding genes typically found in animal mitochondrial genomes. However, in distorter females, the ND6 gene is unusual and contains a stop codon in the middle of the coding region. Although this is uncommon, a functional protein may still be formed despite the presence of the stop codon in this sequence. For instance, in other taxa there is evidence for read through stop codons, codon reassignment, or ribosomal frameshifting (among other variations in protein translation) that result in functional proteins being produced despite peculiarities in the gene sequence that produces them (Baranov et al. 2015). We do not have enough evidence in this case, however, to say whether ND6 in distorter females is translated into a functional protein despite the stop codon, is a pseudogene, or is in a different location that we were unable to identify in the distorter *L.* nr. *bostrychophila* genome.

In addition, as the distorting element is maternally transmitted, it is possible that the mitochondria itself is in some way implicated in the sex ratio distortion that occurs in distorter females. However, the sequence from the normal and distorter *L.* nr. *bostrychophila* mitochondrial do not support this hypothesis. I explored the identity of

any ORF of more than 120bp in both the normal and distorter mitochondrial genomes. I did not find any evidence of an element that may cause sex ratio distortion in the mitochondrial genome of distorter females. Additionally, in plants, the only eukaryotic taxa known to possess mitochondria that distort sex ratio, CMS (cytoplasmic male sterility) causing mitochondria most commonly posses novel genes, often with truncated versions of mitochondrial genes attached to regions coding for novel function (Burt and Trivers 2006). I was unable to find any evidence for this in distorter females. These facts suggest, in corroboration with the evidence that part of the nuclear genome in distorter females is not found in normal individuals, that the distorting element is not likely to be mitochondrial.

Since the genus *Liposcelis* belongs to a lineage with rapidly evolving mitochondria, it is possible that there is intraspecific mitochondrial variation both between the female types and within the female types. I investigated this by amplifying five regions of the mitochondrial genome (most of which spanned more than one gene) in eight individuals of both normal and distorter females and inspecting the PCR band size obtained from these reactions. If the mitochondria differ considerably within a female type, I would expect that either the primers would not amplify any product or that the PCR band obtained would be of a different size than expected. I found that for all eight individuals of each female type, I was able to amplify a band of the expected size. However, laboratory populations were founded by a limited collection of individuals, so it is possible that individuals in the laboratory would have similar mitochondrial conformation due to the limited genetic diversity of the original collection (i.e. the similar mitochondrial sequence within female types would represent a laboratory artefact). The sequence I obtained for the mitochondrial gene CO1 from field caught samples collected years after the original specimen were collected, however, also differed between female types but was very similar for individuals within the same female type (Chapter 4). These findings suggest that there is little mitochondrial polymorphism within different individuals of the same female type. Additionally, these findings give further support that the distorting element is in perfect linkage with the mitochondrial haplotype in distorter

females and also suggests that mitochondrial markers are an accurate method for determining whether an individual is a distorter or normal female.

Finally, I found that the relationship between the different *Liposcelis* species obtained through a phylogeny of mitochondrial genes yields the expected relationship between species based on morphology. As expected, *L.* nr. *bostrychophila* females are most closely related to each other (although with large divergence in the mitochondrial genomes of the different female types). Additionally, the closest relatives to *L.* nr. *bostrychophila* are *L. bostrychophila* and *L. paeta.* This mirrors the grouping *Liposcelis* species are placed into based on morphology (Mockford, 1993). Given that the mitochondrial gene arrangement in the *Liposcelis* sp. with their mitochondrial genomes sequenced is almost entirely different, the manner in which the mitochondria have evolved in this lineage, and in *L.* nr. *bostrychophila* specifically, remains a mystery. In some plant species with multiple mitochondrial chromosomes, it is proposed that recombination between repeat regions in the genome result in multiple chromosomes (Sloan 2013). Since there are several sizable repeat regions in different minichromosomes of each of the mitochondrial haplotypes in *L.* nr. *bostrychophila*, this mechanism may also have caused multiple mitochondrial chromosomes in *L.* nr. *bostrychophila.* Mitochondrial information from a greater diversity of species in this genus, or more information on intraspecific diversity in mitochondrial configuration may provide much needed information to answer questions about the evolution of multiple mitochondrial chromosomes in this genus.

Conclusions

Psocodeans have unusual mitochondria, with multiple mitochondrial chromosomes and rapidly evolving mitochondria found in a number of species within this lineage. In *L.* nr. *bostrychophila,* the presence of the distorting element that manipulates reproduction in distorter females has led to the bizarre and unprecedented mitochondria in normal and distorter females in this species. Not only do the different female types have different mitochondria in terms of gene order, but they also have a different number of mitochondrial minichromosomes, with the gene sequence in these chromosomes being

approximately 25% divergent from each other. This is the first study to relate mitochondrial differences of this magnitude to a selfish genetic element in an animal species, contributing to the body of knowledge about how selfish genetic elements affect the evolutionary trajectory of a lineage as well as opening up new avenues of investigation into how cytonuclear compatibility and mitochondrial function are affected by this level of mitochondrial divergence within interbreeding members of a population.

Supplementary Information for Chapter 3

Supplementary Table 3-1. PCR primers used to sequence the mitochondrial genomes of normal and distorter *L.* nr. *bostrychophila.* Mitochondrial circles are named for the largest gene in the circle. Primer sets used in the investigation of mitochondrial variation within female types are indicated with ** and regions that were unable to be sequenced are indicated with *.

Female Type	Minicircle[1]		Forward Primer	Sequence	Reverse Primer	Sequence	Additional information
Normal	CO1		MCO1F1	GAATTTCCTTTCACCTTCATGG	MCO1R1	ACTAGCAGGTTTCCCACGTC	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
			MCO1F2	AATGGTTCACCCCGTACCTG	MCO1R2	CCCATGAAGGTGAAAGGAAA	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
		**	MCO1F3	GGGGAAATGAGGGATCAAAT	MCO1R3	GTTTTGTCCCGCATAAGGAA	95°C×3min, (94°C×1min, 58°C×1min, 72°C×4min)×35, 72°C×10min
	ND4		MND4F1	TGCAGTCCATGAAAGCCTGT	MND4R1	GATGCTAATCCTGGGCGACT	95°C×3min, (94°C×1min, 52°C×1min, 72°C×1.5min)×35, 72°C×10min
			MND4F2	AGTCGCCCAGGATTAGCATC	MND4R2	ACAGGCTTTCATGGACTGCA	93°C×3min, (93°C×20sec, 60°C×1min, 68°C×5min)×35, 68°C×5 min
			MND4F3	TGGTCTGCAAGATTCCGTTAAAA	MND4R3	CAAGCCCATGACCGTGAAAT	93°C×3min, (93°C×20sec, 58°C×1min, 68°C×3min)×35, 68°C×5 min
	ND1	**	MND1F1	GCTTATCCTCGTTTGCGATT	MND1R1	ACGAAAATTCCATGCCCCCA	95°C×3min, (94°C×1min, 60°C×1min, 72°C×1.5min)×35, 72°C×10min
			MND1F2	CTCCCTTTGATTTGGCAGAA	MND1R2	ACAGGCTCAAGGAGGAATGA	95°C×3min, (94°C×1min, 58°C×1min, 72°C×1.5min)×35, 72°C×10min
	ATP6		MATP6F1	TTACCCGGATATGGATTGGA	MATP6R1	GAACACAAAGGGCAACAACC	95°C×3min, (94°C×1min, 58°C×1min, 72°C×4min)×35, 72°C×10min
			MATP6F2	CAAGGCCGCAATTATGAAAT	MATP6R2	GGGGAATGATCGTGAAAGAA	93°C×3min, (93°C×20sec, 56°C×1min, 68°C×5 min)×35, 68°C×5 min
	ND6		MND6F1	TCCATGACTTTAGAGTTTGAATGAGG	MND6R1	GAAAATGATTTGCGGGAAGA	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
		**	MND6F2	TTCCCGCAAATCATTTTCTC	MND6R2	AAAAGTGATTATGAGGCCACCAA	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
	16S		M16SF1	TGGCGGCTTTTATTCACATT	M16SR1	TGGGGTTACCCTGAACTCAT	95°C×3min, (94°C×1min, 58°C×1min, 72°C×4min)×35, 72°C×10min
			M16SF2	GCCGCAGTAAATTGTGCCAA	M16SR2	CAAACCGCCCGTCACTTCTA	95°C×3min, (94°C×1min, 54°C×1min, 72°C×2min)×35, 72°C×10min
		**	M16SF3	CAAAATTAAGCCGCCAAGAA	M16SR2	CAAACCGCCCGTCACTTCTA	93°C×3min, (93°C×20sec, 59°C×1min, 68°C×3.5min)×35, 68°C×5 min
	ND5	**	MND5F1	CAATGAAGGTGGTATCCCCATA	MND5R1	GTCACCTTTTCTGGCGACTC	93°C×3min, (93°C×20sec, 56°C×1min, 68°C×5 min)×35, 68°C×5 min
		*	MND5F2	CAATGAAGGTGGTATCCCCATA	MND5R2	ATTAATCATGTCAGGCCTAATTTT	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
Distorter	CO1	**	FCO1F1	TAATGCCCAAGTCCGGATGG	FCO1R1	TGCTCACACAATGAACCCCA	93°C×3min, (93°C×20sec, 58°C×30sec, 68°C×6min)×35, 68°C×5min
			FCO1F2	CAACCCCCAAAAACCCATTC	FCO1R2	AATCAACGGGAACACAAGGT	95°C×3min, (94°C×1min, 58°C×1min, 72°C×2min)×35, 72°C×10min
	ND4	**	FND4F1	TAACCGCACTAGAACCCCCA	FCO1R1	TGAACTGGGGCCTCAACATG	93°C×3min, (93°C×20sec, 58°C×30sec, 68°C×6min)×35, 68°C×5min
			FND4F2	TCACATGGGTTTTTATCCCCTTT	FCO1R2	GGAATTTGAGTATGTCCCTTCC	95°C×3min, (94°C×1min, 58°C×1min, 72°C×2min)×35, 72°C×10min
	16S	**	FATP6F1	AGAGTGATTGGAAGGGCAAC	FATP6R1	CATCGAGGTCGCAATCATAA	95°C×3min, (94°C×1min, 58°C×1min, 72°C×1.5min)×35, 72°C×10min
			FATP6F2	ACAGCCGCAGTAAATTGTGC	FATP6R2	TCTAGGCATGTCCTACCCTGA	95°C×3min, (94°C×1min, 58°C×1min, 72°C×1.5min)×35, 72°C×10min
	ND5		FND5F1	CGTGCCTTGTGGAATGGTT	FND5R1	TCGAATATCTTGCCACCCGG	95°C×3min, (94°C×1min, 54°C×1min, 72°C×1.5min)×35, 72°C×10min
		*	FND5F2	CCGGGTGGCAAGATATTCGA	FND5R2	CAACCATTCCACAAGGCACG	93°C×3min, (93°C×20sec, 58°C×30sec, 68°C×6min)×35, 68°C×5min
		**	FND5F3	TGAACGATCTAAAACTCGAAGAA	FND5R2	CAACCATTCCACAAGGCACG	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
	12S		F12SF1	TGCCCTGTTCAAGAAAATTG	F12SR1	TTACTCGGCGAAAGCTTCAT	93°C×3min, (93°C×20sec, 58°C×1min, 68°C×3min)×35, 68°C×5 min
		**	F12SF2	ATGAAGCTTTCGCCGAGTAA	F12SR2	TCGGGTGATAACCTACAACCA	95°C×3min, (95°C×20sec, (63°C-53°C)×30sec, 72°C×1.3min)×10, (95°C×20sec, 55°C×30sec, 72°C×1.3 min)×24, 72°C×5min

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Chapter 4 - Assessing the species and genetic diversity in wild booklice (Psocodea: *Liposcelis*), a lineage with rapidly evolving mitochondria and unusual reproductive systems.

Abstract

Populations containing reproductive manipulators are expected under many conditions to be unstable, especially if they induce a strong sex ratio bias into the population. *Liposcelis* nr. *bostrychophila* is a sexual booklouse species containing a reproductive manipulator (likely a chromosomal selfish genetic element) that causes individuals that carry it to produce only female offspring. This species was originally collected in Arizona in 2010, but given the strong female bias in populations it is uncertain whether this species persists in the wild over time. I conducted a survey to determine whether *L.* nr. *bostrychophila* (and the selfish genetic element it carries) is still present in the Chiricahua Mountains and to investigate the species and genetic diversity of other *Liposcelis* in this region. I found that *L.* nr. *bostrychophila* females that carry the selfish genetic element continue to persist in the wild with females that do not carry it. Additionally, I found that, like *L.* nr. *bostrychophila,* other *Liposcelis* species in the Chiricahua Mountains also exhibit female biased populations sex ratios, suggesting that *L.* nr. *bostrychophila* populations may have had female biased sex ratios before the selfish genetic element arose in this species. Additionally, I found that wild *Liposcelis* species have high intraspecific mitochondrial variation; with the two most abundant groups we sampled (one being *L.* nr. *bostrychophila*) having approximately 20% divergence in the mitochondrial gene CO1 in different members of the same lineage. As the first in depth examination into the species and genetic diversity of wild *Liposcelis*, I uncovered a number of interesting findings on *Liposcelis* diversity and genetics, supporting the importance of field investigations in learning more about this unusual insect group.

Introduction

Insects comprise approximately 70% of documented animal species, and as such are an exceptionally diverse animal clade (Scheffers et al. 2012). Perhaps because of this, several insect lineages have not been investigated in great detail and as a consequence we know relatively little about the ecology and evolution of many insect species. The genus *Liposcelis* is one of these lineages. *Liposcelis* are booklice that belong to the order Psocodea, which contains booklice, barklice, and parasitic lice (or the former orders Psocoptera and Phthiraptera). Booklice are the closest free-living relatives of parasitic lice (Yoshizawa and Johnson 2003). They are small, wingless insects (less than 3mm in length) that are best known as stored grain pests (Mockford, 1993). Booklice in the genus *Liposcelis* have recently been found to have a number of interesting features. *Liposcelis* species have been found to have rapidly evolving mitochondria (Shao et al. 2009, Chen et al. 2014b), several examples of unusual reproductive systems in some cases due to reproductive manipulation by selfish genetic elements, and have female biased sex ratios in at least one lineage (Yusuf et al. 2000, Perlman et al. 2015) (Chapter 2). Despite this, very little research has been conducted on *Liposcelis* species, especially on the wild diversity and ecology of this insect clade (although see Broadhead and Richards 1982, Baz 1991, Mockford and Krushelnychy 2008).

A *Liposcelis* species was recently discovered with an interesting reproductive biology. *Liposcelis.* nr. *bostrychophila* was originally collected in the Chiricahua Mountains, Arizona (approx. 31.9N,109.3W) in 2010 (Perlman et al. 2015). This species is not a stored grain pest and is known only from the collections in the Chiricahua Mountains. *Liposcelis* nr. *bostrychophila* is a sexual species, but harbours a selfish genetic element (designated the distorting element) that manipulates reproduction in some individuals. The distorting element is maternally transmitted and causes individuals that carry it to produce only female offspring (distorter females), while females that do not carry it produce both male and female offspring (normal females) (Perlman et al. 2015). Populations containing selfish genetic elements that manipulate reproduction are expected to often be unstable in their host population, due to the sex ratio bias they induce in their host population (Hamilton 1967, Hatcher et al. 1999). Since distorter *L.* nr.

bostrychophila females produce only females, this species may be particularly unstable (Chapter 2). Therefore, we do not know whether the distorting element (and *L.* nr. *bostrychophila* in general) is able to persist in the wild.

Psocodeans have recently been gaining attention due to their rapidly evolving mitochondria. The mitochondrial genome in animals is generally well conserved, with genes arranged on a single circular chromosome (Boore 1999). However, Psocodea contains many species with bizarre mitochondrial conformations. It is the only animal lineage known to have multiple circular mitochondrial chromosomes (Cameron 2014). The most unusual example of this is the human louse, *Pediculus humanus,* whose mitochondrial genes are arranged on at least 18 mitochondrial minichromosomes (Shao et al. 2009). Several *Liposcelis* species have had their mitochondrial genomes sequenced, with some surprising and interesting results. One species (*Liposcelis decolor*) has a single circular mitochondrial chromosome; several species (*L. entomophila, L. paeta,* and *L. bostrychophila*) have two mitochondrial minichromosomes, and *L.* nr. *bostrychophila* has two reproductive phenotypes with five or seven mitochondrial minichromosomes depending on the reproductive phenotype (Wei et al. 2012a, Chen et al. 2014a, 2014b, Perlman et al. 2015). Additionally, the level of divergence in mitochondrial genes between different *Liposcelis* species is remarkably high (Chapter 3).

The distorting element in *L.* nr. *bostrychophila* has affected its mitochondrion (Chapter 3). Mitochondria are maternally transmitted and since the distorting element is also maternally transmitted, is it in perfect linkage disequilibrium with other maternally transmitted elements (Hurst and Jiggins 2005). In laboratory colonies, the mitochondrial genome of normal and distorter females are approximately 25% divergent (Perlman et al. 2015) (Chapter 3). Additionally, the mitochondrial haplotype of a female is perfectly associated with whether she carries the distorting element and there is little variation in the mitochondrial genome within individuals of the same female type (Chapter 3). However, the individuals used to sequence the mitochondria of normal and distorter females were from laboratory cultures (originally collected in the 2010 field collections), so it is possible that there is greater mitochondrial diversity in wild populations.

Additionally, little is known about mitochondrial diversity within other *Liposcelis* species (although see Wei et al. 2012b), which may be interesting to consider given the elevated rate of mitochondrial evolution within this lineage.

Another interesting aspect of *L.* nr. *bostrychophila* ecology that was recently uncovered is that normal females (who do not carry the distorting element) also produce a female biased sex ratio (Chapter 2). The reason for this is a mystery, although it is not expected to be a result of the presence of the distorting element in this system due to the lack of gene flow from distorter to normal females (since distorter females never pass their genes on to normal females) (Werren 1987). Therefore, it would be interesting to determine whether other *Liposcelis* species also produce female biased sex ratios. Additionally, exploring what factors *Liposcelis* species that have female biased sex ratios have in common to *L.* nr. *bostrychophila* may provide insight into why normal females (and other *Liposcelis* that also have female biased sex ratios) exhibit this unusual trait.

Additionally, *Liposcelis* species have diverse reproductive systems depending on the species. Some species are parthenogenetic while others are sexual, and as discussed, *L.* nr. *bostrychophila* has an unusual reproductive system containing two distinct reproductive phenotypes (Mockford 1971, 1993, Perlman et al. 2015). *Liposcelis bostrychophila*, a close relative to *L.* nr. *bostrychophila*, is a parthenogenetic member of this lineage. In addition, *L. bostrychophila* contains a vertically (maternally) transmitted endosymbiotic bacterium: *Rickettsia felis* (Yusuf et al. 2000, Behar et al. 2010). Although *Rickettsia* species can be reproductive manipulators, increasing the proportion of females in their host's offspring to increase their transmission to future generations (as endosymbiotic bacteria are not passed on from males to their offspring) (Perlman et al. 2006), there is no conclusive evidence that *Rickettsia felis* induces parthenogenesis in *L. bostrychophila. Rickettsia* does not appear to be present in the other *Liposcelis* species surveyed to date (including *L.* nr. *bostrychophila*), however, surveying additional species will shed light on whether this bacterium is always associated with parthenogenesis in this lineage (Perlman et al. 2015).

I conducted a survey of wild *Liposcelis* in the Chiricahua Mountains, Arizona, the original collection site of *L.* nr. *bostrychophila*. I had several goals in undertaking this study. First, I wanted to determine whether I was able to collect *L.* nr. *bostrychophila* in this area four years after the original collection was made, and whether the distorting element (and consequently distorter females) were able to persist in populations over this time period. I also wanted to assess whether the mitochondrial diversity of *L.* nr. *bostrychophila* collected four years after our laboratory collections were made show a similar pattern of mitochondrial diversity as the laboratory cultures, with extreme mitochondrial variation between normal and distorter females but little mitochondrial variation within individuals of the same female type. I also wanted to assess the species and genetic diversity of other *Liposcelis* species in the same habitat as *L.* nr. *bostrychophila*, to gain a better understanding of the species living in the same habitat as *L.* nr. *bostrychophila*. Finally, I screened individuals for the presence of *Rickettsia,* to determine how common this endosymbiont is in *Liposcelis* lineages and to gain a better understanding of whether this endosymbiont is always associated with parthenogenesis. This study will allow us to assess whether an unusual *Liposcelis* species that carries a selfish genetic element that induces a strong female bias in individuals carrying it (and the population in general) is able to persist in the wild. Additionally, this survey will help us to understand more about the diversity and population genetics of wild *Liposcelis* species.

Methods

Sample Collection and PCR

Along with Steve Perlman and Ed Mockford (Illinois State University), I collected wild *Liposcelis* individuals from 9 sites in the Chiricahua Mountains, Arizona in July and August 2014. The collection sites spanned the Chiricahua Mountains from east to west and also included one site outside the Chiricahua Mountains to the east (site 9). We collected individuals using two methods: we sifted leaf litter using a large wire strainer onto a white sheet and collected the individuals falling onto the sheet, and we collected individuals from yucca plants (as well as other vegetation in the area) by beating branches or leaves and collecting the individuals that fell out onto a white sheet.

We grouped individuals by the site they were collected from and stored them in 95% ethanol. I examined the collections from each site and separated individuals into groups based on morphology. I extracted DNA from several individuals of each group as well as all individuals that appeared morphologically to be *L.* nr. *bostrychophila*. I also took a photo and recorded the sex of each individual for which I extracted DNA. I extracted DNA from each individual using 20ul PrepMan Ultra (Life Technologies) (to obtain 10ul of DNA). I amplified an approximately 400 bp region of the CO1 gene (using primers L6625 and H7005) and an approximately 900bp region of 18S (using primers 18Sai and 18Sbi). The PCR conditions for each primer set as well as the primer sequences are listed in Supplementary Table 4-1. I sequenced these regions using Macrogen USA standard sequencing with purification.

I wanted to ensure that I had correctly separated individuals into lineages based on their morphology. In order to assess this, I extracted DNA from ten additional individuals from the lineage that we had collected the most specimens from (Lineage II). I extracted DNA from these individuals and amplified the 18S region using the same technique as for the other specimens. I then compared the 18S region of these individuals to the rest of the specimens collected (using phylogenetics) to ensure they clustered with the group to which I had assigned them based on morphology.

Sequence alignment and phylogenetics

For the nucleotide sequence alignments and phylogenies I used Geneious v6.1 and MEGA 5.1 (Tamura et al. 2011). I analyzed the 18S sequences and the CO1 sequences separately, since I specifically wanted to assess the mitochondrial variation of individuals collected. I included genetic sequence from several additional specimens (in phylogenies) to assess whether any of the individuals collected belonged to these species. I extracted DNA and amplified the CO1 and 18S region from voucher specimens of *L. bostrychophila, L. paeta, L. entomophila, L. decolor, L. brunnea, L. pearmani,* and both distorter and normal *L.* nr. *bostrychophila* using the same protocol as for the field collected individuals. I also obtained genetic sequence from *L. corrodens* and the

booklouse *Lepinotus reticulatus* (Psocodea: Trogiomorpha) (to use as an outgroup in phylogenies) that previous members of the Perlman lab sequenced.

I aligned the 18S sequences using a ClustalX alignment with the default settings. I manually inspected the alignment, then exported it to MEGA (Tamura et al. 2011) and computed the optimal nucleotide substitution model using the default settings. I generated a maximum likelihood phylogeny of these sequences in Geneious using the PhyML package (Guindon and Gascuel, 2003) and using the Kimura 2 parameter nucleotide substitution model, a combination of NNI and SPR topology searches, and 500 bootstrap replicates. NUMTs, or stretches of mitochondrial sequence inserted into the nuclear genome, are present in many eukaryotes (Richly and Leister 2004). Therefore, before aligning the CO1 sequences, I assessed whether sequences were NUMTs or part of the mitochondrial genome. I did this by ensuring that the sequence obtained from the PCR reaction was of the correct size, examining the sequence chromatogram to check for multiple peaks at the same locus, and translating the sequence to ensure that there were no stop codons in the region sequenced (since this would indicate the sequence would not be able to give rise to a functional protein). I used a MUSCLE translation alignment to align the sequences with a gap penalty of -1.2. I manually inspected the alignment, then exported it to MEGA (Tamura et al. 2011) and determined the optimal nucleotide substitution model. I assembled a maximum liklihood phylogeny in Geneious using the PhyML package (Guindon and Gascuel, 2003). I used a general time reversible nucleotide substitution model, a combination of NNI and SPR topology searches and 500 bootstrap replicates to generate the phylogeny.

Rickettsia screening

I screened for the presence of *Rickettsia* using the 16S primer set RSSUF and RSSUR (Supplementary Table 4-1). I screened one individual from each lineage (using the 18S phylogeny) in duplicate for the presence of *Rickettsia*. If the individual was positive for *Rickettsia,* I screened the other individuals within the same lineage for the presence of *Rickettsia* using the same primer set and protocol. I then sequenced the PCR products of the *Rickettsia* positive individuals.

Results

Phylogenetic relationship of wild Liposcelis individuals

I collected a total of 361 *Liposcelis* individuals from the Chiricahua Mountains, Arizona, in 2014 (Table 4-1). Wild caught individuals belonged to seven different lineages (Figure 4-1, 4-2). For one of the lineages (Lineage V), I was only able to amplify sequence from the 18S region (Figure 4-1) while for another lineage (Lineage VII) I was only able to amplify sequence from the CO1 region (Figure 4-2). Photos of a female (and a male if possible) of each lineage are included in the supplementary information (Supplementary Figures 4-1 to 4-6). The majority of the *Liposcelis* individuals collected belonged to Lineage II (309 of 371 individuals). Additionally, very few wild males were collected, but those that were collected belonged to Lineages II and III, indicating that these species are sexual. I was unable to amplify either 18S or CO1 sequence from five individuals. These individuals were not included in phylogenetic analyses. All CO1 sequences were of the expected size and translated into a functional region of the CO1 gene (i.e. they did not contain stop codons in the region sequenced) suggesting that these sequences are part of the mitochondrial genome and are not NUMTs. I amplified the 18S region from ten additional individuals from Lineage II to ensure that I correctly separated individuals into lineages based on morphology. These individuals all clustered in Lineage II, suggesting that segregating individuals based on morphology was an accurate method of separating genetically distinct individuals.

Table 4-1. Summary of female and male *Liposcelis* collected from Chiricahua Mountains, Arizona, 2014. Lineages indicate individuals that are distinct based on morphology and sequence divergence (with Lineage 1 representing the *L.* nr. *bostrychophila* specimens collected). Numbers indicate the total number of individuals collected that were identified based on morphology with the numbers in parentheses indicating the number of individuals within this lineage that were identified by molecular means as well.

Lineage	Female	Male	Total
I (*L*. nr. *bostrychophila*)	19(15)		19
II	276(33)	33(12)	309
III	12(4)	2(1)	14
IV	1(1)		1
V	1(1)		1
VI	1(1)		1
VII	10(3)		10
Unknown	3(3)	3(2)	6

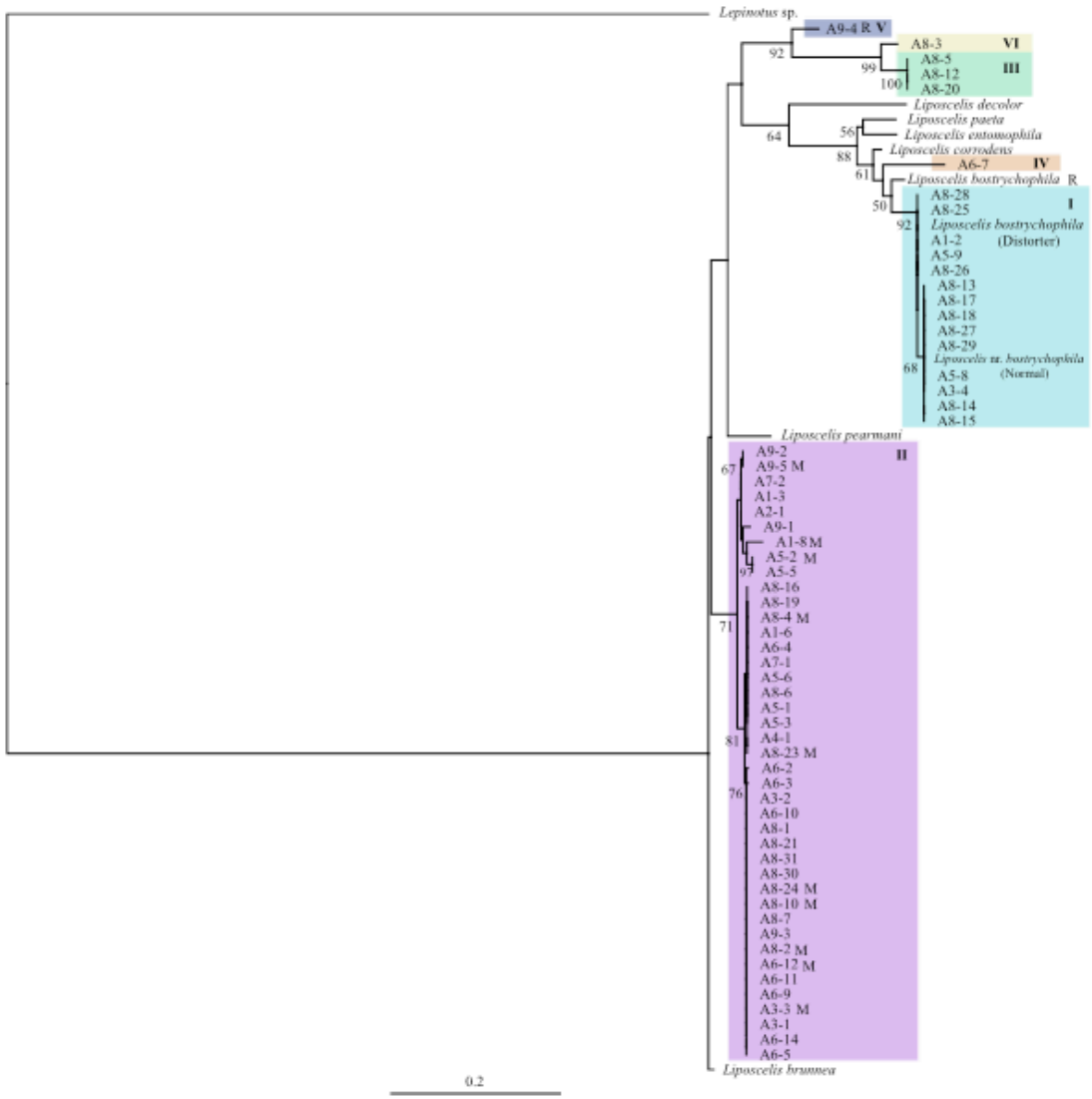

Figure 4-1. Maximum likelihood phylogeny of *Liposcelis* individuals collected in Arizona 2014, based on the nuclear ribosomal gene 18S. Specimens collected are identified with A (for Arizona) followed by the site they were collected from (1-9) and the individual collected from that site. Lineages are identified with coloured boxes. Males are identified with an M beside the individual identifier and individuals that carried *Rickettsia* have an R placed beside the individual identifier. Nodes with bootstrap support above 50 are indicated. The phylogeny was generated using a Kimura 2 parameter nucleotide substitution model and 500 bootstrap replicates.

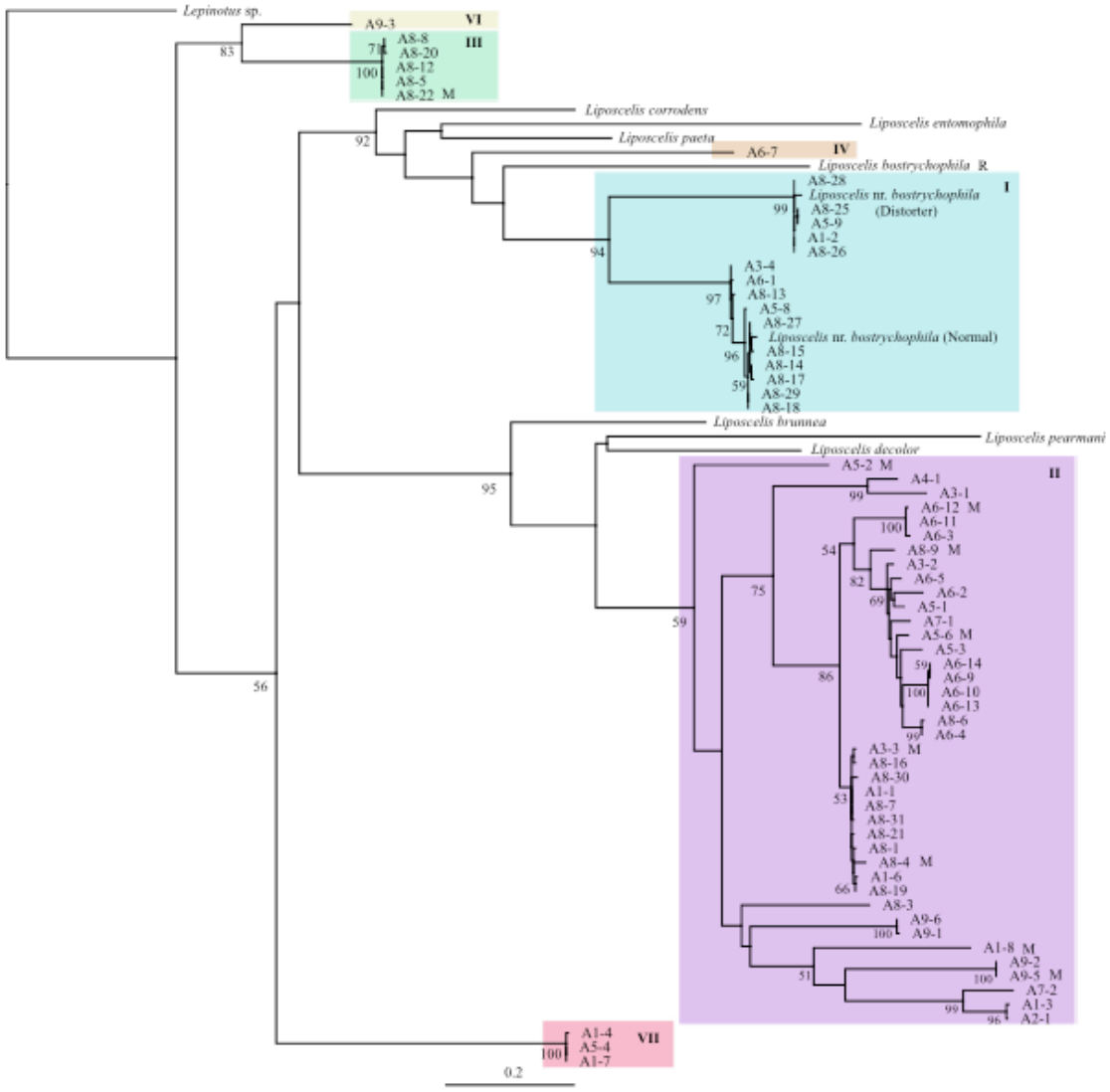

Figure 4-2. Maximum likelihood phylogeny of *Liposcelis* individuals collected in Arizona 2014, based on the mitochondrial gene cytochrome oxidase 1. Specimens collected are identified with A followed by the site they were collected from (1-9) and the individual collected from that site. Lineages are identified with coloured boxes. Males are identified with an M beside the individual identifier and individuals that carried *Rickettsia* have an R placed beside the individual identifier. Nodes with bootstrap support above 50 are indicated. The phylogeny was generated using a general time reversible nucleotide substitution model and 500 bootstrap replicates.

The phylogeny generated using CO1 sequence had a similar topology to that generated using 18S sequence. However, the amount of variation in CO1 sequences was much larger than for 18S, both within lineages and between lineages. I collected 19 individuals that were morphologically and/or genetically identified as *Liposcelis* nr. *bostrychophila*. All of these individuals were female. Ten of these individuals were identified as normal females and five were identified as distorter females based on the CO1 sequence (I was unable to amplify the CO1 region from four of the individuals). Additionally, there was very little mitochondrial variation between individuals of the same mitochondrial haplotype.

I screened a representative from each lineage for the presence of *Rickettsia*. I found that the individual from Lineage V as well as one of the individuals from Lineage II (A1-1) were positive for *Rickettsia* using the 16S primers RSSU F/R; these two sequences were 98% similar to each other. I screened the remainder of the individuals in Lineage II for which I extracted DNA with the RSSU F/R primers and found that none of these individuals tested positive for *Rickettsia*. BLAST searches (National Center for Biotechnology Information) revealed that the sequences obtained from both individuals were 99% similar to the *Rickettsia* from *L. bostrychophila*.

Discussion

Liposcelis nr. *bostrychophila* is an unusual species that carries a selfish reproductive manipulator that causes individuals that carry it to produce exclusively female offspring. The presence of a reproductive manipulator that induces a strong sex ratio biases in its host is expected under many conditions to cause the population it resides in to be unstable (Werren 1987, Hatcher et al. 1999). I undertook one of the first investigations into the abundance and diversity of wild *Liposcelis* species. My aim was to assess whether *L.* nr. *bostrychophila,* which was originally collected in the Chiricahua Mountains in 2010, could still be found in this region and whether *L.* nr. *bostrychophila* populations still contained both normal females and distorter females (which carry the distorting element). I also wanted to investigate other *Liposcelis* species in this region and

assess how these species compare to *L.* nr. *bostrychophila* in terms of their population sex ratio and population genetics.

I was able to collect a total of 19 *L.* nr. *bostrychophila* individuals from the Chiricahua Mountain. I collected individuals from five of the nine sites we collected *Liposcelis* from, and identified both normal and distorter females in three of the five sites we collected this species from. The fact that we were able to collect both distorter and normal females indicates that both female types have coexisted in the wild from 2010 to 2014. Although a four year period is not an exceptionally long time, *L.* nr. *bostrychophila* can produce several generations per year and theoretical studies indicate that selfish genetic elements inducing a 100% female bias in their host are inherently unstable in populations unless there are other factors mediating their persistence (i.e. fitness costs etc.) (Werren 1987, Hatcher and Dunn 1995). The fact that both female types seem to be able to persist in the wild gives support to the findings from my laboratory experiments assessing the ecological factors allowing for the persistence of the distorting element (Chapter 2). In these experiments I found that normal females produce a female biased sex ratio and distorter females have a lower fitness than normal females, mediating the persistence of the distorting element in laboratory settings (Chapter 2). Additionally, I found that although the frequency of the distorting element in laboratory populations of *L.* nr. *bostrychophila* changed in populations over time, it was also able to persist for many generations. The persistence of normal and distorter *L.* nr. *bostrychophila* in wild populations suggests that these factors are important in the persistence of this species in the wild as well as in laboratory settings.

Interestingly, I found that there was a female bias in all of the lineages for which we were able to collect males (with 11% and 17% of individuals collected being male in lineages II and III respectively). I previously found that normal *L.* nr *bostrychophila* females produce a female biased sex ratio (with only approximately 35% of a normal female's offspring being male) (Chapter 2). Although it is possible that fewer males were collected because males do not live as long as females or may be more difficult to collect than females, it seems that several sexual *Liposcelis* species likely produce female biased

sex ratios. Therefore, The female bias in *L.* nr. *bostrychophila* may have been present before the distorting element arose in this population. If this is the case, it is interesting to consider whether the female bias in this population had an impact of the evolution of the unusual reproduction system in this species. The field collections and laboratory studies on *L.* nr. *bostrychophila* suggest that the frequency of males in populations is extremely low (Chapter 2). We know little about how *L.* nr. *bostrychophila* populations are able to persist over time, given the low frequency of males. This would be a fruitful area of future research. However, due to the widespread female bias in *Liposcelis* populations, it seems that *L.* nr. *bostrychophila* populations were likely able to persist with a low frequency of males in populations before the distorting element arose in this species. Therefore, the persistence of the distorting element in this system may have been aided by the female biased sex ratio in *Liposcelis* populations, since this species was likely already able to cope with a female bias sex ratio in populations when the distorting element arose. There are no studies to my knowledge that have investigated whether a female biased population sex ratio makes it more likely that a selfish genetic element that biases the sex ratio of individuals towards females is able to persist in populations. Further investigation into this topic may be warranted.

Liposcelis species have rapidly evolving mitochondria (Wei et al. 2012a, Chen et al. 2014b). In other lineages with rapidly evolving mitochondria, the level of intraspecific mitochondrial variation depends on the species in question. For instance, *Tigriopus californicus,* a copepod species with rapidly evolving mitochondria, has a high level of intraspecific mitochondrial divergence (more than 18%) (Burton and Lee 1994), although *Nasonia* wasps (which also have rapidly evolving mitochondria) have low levels of intraspecific mitochondrial divergence (less than 2%) (Van Opijnen et al. 2005). The low level of intraspecific mitochondrial variation in *Nasonia,* however, may reflect that this lineage carries several strains of *Wolbachia,* which may have reduced intraspecific mitochondrial variation due to cotransmission with the endosymbiont (Van Opijnen et al. 2005).

In *L.* nr. *bostrychophila,* the level of intraspecific mitochondrial divergence is similar to what is observed in *Tigriopus californicus,* with distorter females and normal females having distinct mitochondrial haplotypes that are more than 25% divergent at the nucleotide level (Perlman et al. 2015). In this field collection, I found that although females of different female types have very different mitochondrial haplotypes (with approximately 20% divergence in the region of CO1 sequenced), there is little variation in the mitochondrial sequence between females of the same female type. Lineage II (the most abundant species we collected) also had a high level of mitochondrial diversity between individuals that was similar in scale to what was found for *L.* nr. *bostrychophila* (approximately 20% divergence). However, where *L.* nr. *bostrychophila* had two mitochondrial haplotypes, there were many mitochondrial haplotypes in Lineage II. This difference is likely due in part to the presence of the distorting element in *L.* nr. *bostrychophila*. The distorting element has affected mitochondrial evolution in *L.* nr. *bostrychophila* due to the fact that it is transmitted in the same way as mitochondria, and is therefore in perfect linkage with mitochondria (Hurst and Jiggins 2005, Perlman et al. 2015). In other systems with maternally transmitted reproductive manipulators, the presence of the reproductive manipulator often decreases mitochondrial diversity in the population (Ballard et al. 1996, Jiggins 2003, Shoemaker et al. 2004). Therefore, it is possible that *L.* nr *bostrychophila* has fewer mitochondrial haplotypes than Lineage II due to the presence of the distorting element.

Apart from *L.* nr. *bostrychophila,* we collected individuals from six other *Liposcelis* lineages. Interestingly, these lineages did not cluster with any of the voucher specimens in either the 18S or CO1 phylogeny, suggesting that these lineages are not the same species as any of the voucher specimens. This suggests either that I did not have access to genetic information from the species to which these individuals belong or that these lineages have not previously been characterized (and belong to new species). It would not be very surprising if some of the individuals collected belonged to previously undocumented species, since few researchers have attempted to characterize the diversity of wild *Liposcelis* species in this area. However, some of the lineages morphologically resemble characterized species that I did not have access to molecular information from

(for instance, the individual from Lineage V resembles *Liposcelis deltachi*) (Mockford 1993). Further molecular and morphological examination of these individuals would be useful for establishing whether they belong to already characterized species or represent new, undocumented species.

Finally, I also screened for the presence of *Rickettsia* in each of the lineages I identified. I found that the only individual collected from lineage V (a female), as well as one of the individuals screened from lineage II (a female), contained *Rickettsia*. However, given the fact that only one individual was collected from lineage V and the individual was a female, it is impossible to say anything about whether this species is parthenogenetic and whether the *Rickettsia* in this lineage has any role in reproduction in its host. Lineage II is clearly sexual, since males were collected. However, since only one individual within this lineage carried *Rickettsia* and this individual was also a female, I do not have enough evidence to speculate on whether *Rickettsia* in this lineage manipulates host reproduction. Therefore, although I can say that there are *Liposcelis* lineages other than *L. bostrychophila* that contain *Rickettsia,* I do not have enough information to comment on whether *Rickettsia* is associated with parthenogenesis in other *Liposcelis* lineages (Yusuf et al. 2000, Behar et al. 2010).

Conclusion

Liposcelis is a fascinating genus of insects that we know very little about. I undertook one of the first field studies to investigate *Liposcelis* species and genetic diversity, with an emphasis on *L.* nr. *bostrychophila*. I found that *L.* nr. *bostrychophila* is still present in the Chiricahua Mountains, and that both normal and distorter females still persist within *L.* nr. *bostrychophila* populations. Additionally, I found that like *L.* nr. *bostrychophila*, other *Liposcelis* species also exhibit a female biased sex ratio. Additionally, I found that like *L.* nr. *bostrychophila,* the most abundant species I collected (Lineage II) also has high intraspecific mitochondrial diversity. However, the number of mitochondrial haplotypes in this species was larger than for *L.* nr. *bostrychophila,* which has two mitochondrial haplotypes identified to date. Finally, several of the individuals I collected carry the endosymbiont *Rickettsia,* although it is

uncertain whether it is associated with parthenogenesis in these individuals, like in *L. bostrychophila.* I uncovered a number of fascinating pieces of information about *L.* nr. *bostrychophila*, contributing to our knowledge about the distribution and abundance of this species, as well as increasing our knowledge of *Liposcelis* species in general. This will help to establish what ecological factors are associated with the reproductive manipulator in *L.* nr. *bostrychophila,* and which factors are more general for *Liposcelis* species.

Supplementary Information for Chapter 4

Supplementary Table 4-1. PCR primers and thermocycling conditions used for wild *Liposcelis* sequencing.

Region	Primer	Sequence	PCR conditions
mitochondrial - CO1	L6625	CCGGATCCTTYTGRTTYTTYGGNCAY	95°C×3min, (94°C×30sec, 54°C×30sec, 72°C×1.25min)×35, 72°C×3min
	H7005	CCGGATCCACNACRTARTANGTRTCR	
nuclear-18S	18sai	CCTGAGAAACGGCTACCACATC	93°C×3min, (93°C×20sec, 57°C×30sec, 68°C×3min)×40, 68°C×5min
	18sbi	GAGTCTCGTTCGTTATCGGA	
Rickettsia-16S	RSSUF	GCTTTCAAAACTACTAATCTA	95°C×3min, (95°C×1min, 51°C×45sec, 72°C×1min)×30, 72°C×5min
	RSSUR	AAAAGCATCTCTGCGATCCG	

Supplementary Figure 4-1. Female *L.* nr. *bostrychophila* (Lineage I) collected in Arizona, 2014.

Supplementary Figure 4-2. Female (left) and male (right) *Liposcelis* individuals from Lineage II collected in Arizona in 2014.

Supplementary Figure 4-3. Female (left) and male (right) *Liposcelis* individuals from Lineage III, collected in Arizona in 2014.

Supplementary Figure 4-4. Female *Liposcelis* individual from Lineage IV collected in Arizona 2014.

Supplementary Figure 4-5. Female *Liposcelis* individual from Lineage V collected in Arizona 2014.

Supplementary Figure 4-6. Female *Liposcelis* individual from Lineage VI collected in Arizona 2014.

Supplementary Figure 4-7. Female *Liposcelis* individual from Lineage VII collected in Arizona 2014.

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