SEXUAL CONFLICT AND THE EVOLUTION OF NUPTIAL FEEDING

by

JOSEPH RYAN JALINSKY

B.S., University of Kansas, 2012
B.G.S., University of Kansas, 2012

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2014

Approved by:
Major Professor
Dr. Jeremy L. Marshall

Copyright

Abstract

Males providing females with gifts in order to mate is not a novel occurrence. Indeed, depending on the taxonomic system, males may provide gifts ranging from dead insects, to nutritious ejaculates to even subjecting themselves to cannibalistic feeding. Interestingly, while the burden of the costs of these gifts is primarily carried by the male, net fitness of providing or receiving such gifts must be positive for both sexes, if these gifts are to be maintained in a population. If this is not the case, and sexual conflict has exerted a cost-benefit imbalance between the sexes, then the expectation is that the system will evolve towards the reduction of nuptial gift giving. Here, utilizing the *Allonemobius socius* complex of crickets where females benefit greatly from cannibalizing male blood as a nuptial gift, we explore the possibility that sexual conflict is acting on gift size. To do this, we assess the gift-size distribution, and their associated fitness functions, for twelve populations that span the phylogeny and geographic distribution of this complex. We find that gift-size distributions are shifted towards small or no gifts in the majority of populations. Moreover, fitness data suggest that males providing a small or even no gift are equally successful to their large gift-giving counterparts. Taken together, the population profiles indicate that at least half of these populations are evolving towards the near or complete loss of these cannibalistic gifts – a predicted, but previously undocumented, outcome of sexual conflict. We also assess the speed at which sexual conflict is acting to alter gift size distributions in populations by comparing gift sizes between different time points in populations and across phylogenetic history. The implications of these results are discussed relative to antagonistic coevolution of behavioral traits and sexual conflict theory in general.

Table of Contents

List of Figures

Acknowledgements

I would like to thank my major advisor, Dr. Jeremy Marshall, for his guidance throughout my time at Kansas State. He provided me with an invaluable philosophical groundwork upon which to base the way I view and pursue science. I would also like to thank my committee members, Dr. James Nechols and Dr. Mark Ungerer for their support and expertise related to my project. My lab members, Bettina Janke, Desiree Harpel, Larry Rodriguez and Klara Zurek were incredibly helpful and an important part of my graduate school experience. Finally, I would like to thank my wife, Emily Jalinsky, for her patience and support.

Chapter 1 - Making Sense of Sex in the Light of Sexual Conflict

Evolving Perspectives on Sexual Conflict

Like most fundamental concepts in biology, the contemporary view of sexual conflict has been shaped by a history of revision and reinterpretation; as even today, a precise, all-encompassing definition is elusive. Sexual conflict, while not explicitly stated as such, was discussed after Darwin's views on sexual selection had reached the scientific community; it had at least been understood that there was the potential for a difference between the interests of males and females with respect to reproduction. However, it was not until Trivers (1972) published his 'Paternal Investment and Sexual Selection' that the concept of sexual conflict began to gain traction and interest as an important process in evolutionary biology. Trivers correctly rooted his views on conflict in anisogamy (i.e., dissimilar gametes), which leads to the sexes adopting different roles based on gametic investment. He made a series of observations in *Drosophila* that describe general differences between the sexes. First, variation in reproductive success is much higher for males than it is for females. Second, attracting mates is not a limiting factor for females but could be for males. Finally, and most importantly, male reproductive success is positively and linearly correlated with the number of copulations performed, while female reproductive success reached an optimum at fewer copulations than that of males. Succinctly stated - reproductive success in males is limited by his ability to copulate, not by his ability to produce sperm. Conversely, female reproductive success is limited by her ability to produce eggs, not by her ability to find mates.

Based on these observations Trivers reasoned that an extension of anisogamy and its effect on sexual interactions between males and females would result in sex-specific differences in parental investment. Specifically, the parent that invests the greatest amount of resources will be the most discriminating in choosing mates as they have the most to lose. The range of investments are potentially wide, from investing only the gamete (i.e., fertilize, then desert), to complete parental care of the offspring until reproductive age. Females are generally viewed as the greater discriminating, investing sex, as there is a greater investment in eggs as compared with sperm; although role reversals have been observed in nature whereby males are more discriminating (intuitively, discriminating males show the hallmarks of being choosy - i.e., less ornamentation than their female counterpart). Trivers summarized his findings by stating that because of differences in paternal investment each sex is vulnerable to adaptations in the other sex to increase its fitness. The sex with the higher paternal investment is vulnerable to desertion, while the sex with the lower paternal investment is vulnerable to cuckoldry. Trivers concludes with a compelling insight about how sexual conflict operates, “Each vulnerability has led to the evolution of adaptations to decrease the vulnerability and to counter-adaptations”.

Parker (1979) took Trivers’ observations and expanded upon them by placing conflict in genetic and game theory models. Parker analyzed conflict through the lens of economics, namely ascribing costs and benefits to potentially sexually antagonistic traits and how those traits would persist within a population. His genetic model mathematically demonstrated how a sex-limited mutation arising in a male which increased his fitness but was costly to a female could spread in a population. Interestingly, the allele could not only be costly to the female he mates with, but could suppress population-level fitness. Parker showed that females could attain indirect benefits from such male traits as her sons could have the advantageous allele (i.e., sexy sons). The model suggests that it is the degree of the cost to the female (and thus the males), that will determine its ability to persist in a population.

Parker's game theory model, influenced by Maynard Smith (1974), focused on direct costs and benefits, ignoring genetic benefits. The 'War of Attrition' model analyzed interactions as asymmetric strategies for males to either persist or not persist, and for females to either resist or be passive in mating. The outcome of such male-female interactions depend on the differences in the rate of evolutionary adaptation between the sexes, the effectiveness of counter-adaptations to the other sex, and the initial conditions of the contest. In this 'contest', the cost of a particular strategy is dependent on the opposite sex, (i.e., if a non-persistent male encounters a nonresistant female the payoffs are positive for both, but the cost of a male being persistent will only pay off if he encounters a resistant female). Parker's model posits that the state of a male or female during reproductive encounters can lead to evolutionary chases (adaptions and counter-adaptations) that could result in unresolvable evolutionary fluctuations mediated by sexual conflict that are unable to achieve evolutionary stability. The expectation is that outcomes of sexual interactions determined by adaptations such as specialized morphological traits or behavioral strategies are cyclical and stability would not be achieved due to the variety of possible strategies and counter-strategies the sexes may employ.

Both Trivers' and Parker's seminal publications laid the groundwork for invigorating empirical and theoretical research into the dynamics of sexual conflict, but ambiguity as to the detection, description and outcome of conflict has been problematic since. Examples range from debates over whether there can be said to be a 'winner' in sexual conflict (Getty, 1999; Rice & Holland, 1999) to different experimental and conceptual approaches to analyzing conflict (Cameron et al., 2003; Chapman et al., 2003; Cordero & Eberhard, 2003). The most exacting debate is supplied by Arnqvist (2004) in which he criticizes a common mistaken assumption about sexual conflict. He points not only to a publication (Pizzari & Snook, 2003), but to a general audience in which, he perceives, sexual conflict is being misunderstood. The misconception is rooted in the assumption that male and female reproductive fitness are not dependent on one another; that a male, through sexually antagonistic coevolution, lowers the fitness of a female to his gain. Arnqvist points out that male and female fitness cannot evolve independently and that males gain their fitness *through* the female. A male that subverts a female and causes a reduction in her fitness will indirectly lower the population fitness, although the male may still have higher fitness than *other males* in the population. In a population that has a 1:1 ratio of males to females the average fitness of both sexes is equal. Arnqvist describes a common outcome of sexually antagonistic coevolution. A male allele arises that increases his chance of mating compared with the *other males around him* and reduces the average female (and male) fitness. A female allele then arises that counter acts or reduces the effect of the male allele. Now pressure is back on the male to counteract the female's counter-adaptation and so on. A byproduct of sexually antagonistic coevolution is that male and female fitness will fluctuate equally and that over time, because of adaptational load, population fitness will decline, but males cannot alter female fitness without affecting population fitness. For this reason, it is important to realize that sexually antagonistic coevolution is not the male fitness versus female fitness, but instead competing male strategies and competing female strategies within the sexes that shapes population-level fitness and the evolutionary trajectory of sexual conflict.

Sexual Conflict: Phenotype and Evolution

After Parker's publication, interest in sexual conflict exploded as seen by the exponential growth in the number of citations his paper received over the proceeding years (Arnqvist & Rowe, 2005). Researchers were finding that sexual conflict was ubiquitous across plant and animal systems; unexplained behaviors and morphologies could be made sense of only by viewing them through the lens of sexual conflict. A large body of research in insects concerned the grasping and anti-grasping reproductive morphologies of males and females, respectively. These traits can be viewed as the morphological analog to Parker's (1979) persistence and resistance game theory model, where males have evolved complex features to keep a female in place during mating and females have in turn evolved features to resist mating. For many insects, males readily mate multiply; but for females, superfluous matings can be detrimental to life-span and they may resist male advances after a successful copulation (Arnqvist & Rowe, 2005). In water striders, males have evolved clasping genitalia that secure females during mating and females have evolved abdominal spines that resist males (Arnqvist & Rowe, 2002). It was presumed that water striders were in the midst of a sexually antagonistic arms race over the level of 'armament' of each of the sexes, with adaptations that enhance a male's ability to mate with females being met with female counter-adaptations to resist. In a comparative study, researchers found that in species in which male armament was more exaggerated, mating rates were higher compared with species in which female armament was more exaggerated, while a similar degree of armament between the sexes showed an intermediate mating rate (Arnqvist & Rowe, 2002). This suggests that some species are at opposite ends of a sexual conflict continuum that fluctuates due to the level of adaptation of a particular sex at a particular time. Morphological adaptations as an outcome of conflict similar to those in water striders are seen in a wide range of insects including beetles and bed bugs (Carayon, 1966; Aiken, 1992; Aiken & Khan, 1992; Reinhardt et al., 2003).

Studies are not limited to morphology though, as infanticide is a common behavioral strategy for males of social mammals and some arthropods. Males will consume the unrelated young of a population for resources, to eliminate resources for competitors, or to gain access to a female that was reproductively unavailable due to offspring care-giving (Hrdy, 1979). This strategy is obviously costly for females and they have developed counter strategies in several systems that include aggressiveness toward males, ambiguous paternity (polyandry) so male paternity is uncertain, and altering reproductive phases as to not coincide with the presence of infant killing males (Hrdy, 1979; Hiraiwa-Hasegawa, 1988; van Schaik & Janson, 2000). These examples show only a fraction of the extent to which sexual conflict has influenced coevolution of males and females. Conflict has been demonstrated to be a force throughout the mating experience from pre-copulatory exploitation of sensory biases (Sakaluk, 2000) in which males exploit a females pre-existing bias, namely feeding, to postcopulatory conflict in which males load their ejaculates with glandular products that effect female behavior and physiology (Chen, 1996; Chapman, 2001; Wolfner 2002).

The variation of phenotype in extant species mediated by sexual conflict is clearly widespread and varied. It was Parker (1979) who initially proposed that the phenotypic responses observed currently are the outcome of a long evolutionary history; conflict has not only been shaping the role of the sexes since their inception, but was in fact the instigator of sexuality, ‘The primordial sexual conflict concerned the establishment of the sexes themselves’. (Parker, 1972). Imagine the ancestral condition of isogamy, where equal sized gametes would fuse and the cytoplasmic volume of the zygote is the total volume of both gametes added together. Presumably, a higher zygotic volume (resources) would infer higher fitness. However, if volume was independent of fitness then selection would favor an increase in number of gametes, not size. Instead, if volume were a function of fitness, then there would be direct selection on gametes to increase their cytoplasmic reserves and these zygotes would fare better than those whose fusion was with smaller gametes. The propagation of larger gametes has two important consequences: larger gametes would be relatively static as compared to smaller gametes and the smaller gametes would be selected to not only minimize their cytoplasmic contribution down to primarily nuclear material (creating the pressure to increase gamete number, not size), but to, in effect, ‘parasitize’ the larger, relatively sessile gametes. Once a ‘division of labor’ is established the result is a landscape where small gametes (now sperm) can flood the gamete pool while the larger gametes (now eggs) have to compensate with larger volumes to maintain zygotic vigor. Eggs would benefit from fusing with other eggs instead of sperm, but the selected advantage of increased number and motility of sperm would make egg-egg fusion less and less likely over time. In essence, the evolution of anisogamy is directly tied to paternal investment at the level of the gamete, with one gamete selected to reduce resource investment while the other must compensate by investing more. Ultimately, the evolution of anisogamy resulted in a snowball effect that would effectively differentiate gametes further and further to the point where, over time, gamete size would not only predict a sex role but influence sex role evolution in multicellular organisms. Theoretical models have been developed with respect to the evolution of anisogamy and their results are consistent with Parker’s predictions (Bulmer & Parker, 2002; Maire & Doebeli, 2001).

Over biological history, sexual conflict has played, and continues to play, an integral part in the evolution of organisms. The ubiquity of conflict (Rice, 2013) is becoming more and more apparent, which should not be surprising; any system that exists in which individuals have non-identical genomes sets the stage for conflict to act. Studies continue to establish that conflict has had an active role in important evolutionary milestones throughout history - from the evolution of sex chromosome dimorphism in which sexually antagonistic alleles linked to a sex determining gene suppresses recombination and, in turn, generates non-homologous, sex determining chromosomes (Rice, 1996), to the evolution of reproductive barriers (speciation) driven by sexual conflict (Arnqvist et al., 2000; Gavrilets, 2000; Gavrilets & Waxman, 2002). In short, sexual conflict is, at least in modern science, an undervalued force that is responsible for much of the diversity of life seen today.

There remain many unanswered questions, including understanding the strength and direction of sexual conflict acting on organisms. The strength of sexual conflict is difficult to measure; metrics are at the mercy of the extent in which a particular population is experiencing sexual antagonism and conflict can be hidden by populations that are in flux away from extremes. The strength of selection has been explored but is by no means fully understood due to the inherent variability of conflict. Furthermore, the direction conflict is trending toward is rarely demonstrated, although such information could say something about the nature and selective landscape limits on which conflict can act. Finally, comparative approaches that assess conflict across populations and species are lacking. Specifically, are there any predictions that can be made when the conflict dynamics of meta-populations or species are studied that could help determine how strongly conflict can act on a population?

Chapter 2 - Sexual Conflict Mediates Fluctuations in the Size of Cannibalistic Nuptial Gifts

Introduction

Males providing females with gifts in order to mate is not a novel concept; indeed, such behavior can be found in taxa ranging from humans to insects (Boggs, 1995; Thornhill & Alcock, 1983; Vahed, 1998; Kruger, 2008). However, there is extreme variation in the types of gifts, ranging from dowry gifts in humans (Goodly, 1976) to dead prey items in dance flies and birds (Kessel, 1955; Pizzari, 2003), to offerings of cannibalistic feeding in crickets (Eggert & Sakaluk, 1994) and preying mantids (Lawrence, 1992). From these brief examples it is clear that males can go to extreme, even seemingly deleterious, lengths to secure mates; but are the costs always worth it?

From the perspective of the male, the costs associated with nuptial gifts can be extraordinarily high and vary in their form. For example, some males of birds and insects, like those of the great grey shrike (Tryjanowski & Hromada, 2005) and hanging fly (Thornhill, 1980), respectively, forage for prey-item gifts prior to copulation which not only represents an added energy expenditure but a much higher risk of predation compared with females. Males may also provide gifts derived from their own body. Such gifts can be manufactured by the male, as is the case with the spermatophylax in male bushcrickets (Lehmann, 2012), or directly cannibalized from the male, as with blood-feeding in ground crickets (Fedorka & Mousseau, 2002a). Each of these male-derived gifts can be costly, as the spermatophylax can comprise up to 30% of the male's body mass (Lehmann, 2012), while females can consume up to 8% of a male's body mass during blood feeding (Fedorka & Mousseau, 2002a); in fact, some katydid females have come to rely entirely on the male's gift, thus relieving her of the energy expenditure of finding her own food (Voigt, et al., 2005). Finally, in extreme cases, females will cannibalize most, if not all, of the male's body (reviewed in Vahed, 1998). All of these represent significant costs that must be overcome if such gifts are to be beneficial for males.

So, what are the benefits of these gifts to males? In general, the benefits of nuptial gifts are considered to come in the form of mating effort and/or paternal investment. Under a mating effort scenario, gift-giving males benefit by either being allowed to copulate or by increasing the proportion of eggs they fertilize. Under paternal investment, gift giving increases the chances that a male's own offspring survive (Simmons & Parker, 1989). While there is no unanimous agreement on the extent and exclusivity to which each of these alternatives have influenced the evolution of nuptial gifts (Simmons & Parker, 1989), each has been demonstrated as an effective male strategy (Wedell, 1993; Simmons, 1990). However, for these gift-giving strategies to be maintained in a population, the benefits must outweigh costs.

The fact that we observe nuptial gifts in wild populations suggests that for at least some populations the benefits do outweigh the costs for males. Moreover, while there are certainly costs of receiving the nuptial gift for females (Vahed, 2007), such gifts are generally considered to be beneficial for females (Gwynne, 2008). Therefore, if nuptial gift giving is to be maintained in a population, then the net fitness of providing or receiving gifts must be positive for both sexes. If, on the other hand, gifts represent a cost sink for both sexes, then that population should quickly evolve towards the loss of nuptial gifts. In both cases, the net benefits or costs, for both sexes should be equal, or nearly so, with sexual selection being the most likely evolutionary process underlying the maintenance or loss of nuptial gifts, respectively.

Alternatively, there is the case of cost-benefit dynamics being significantly different between the sexes, thus setting the stage for sexual conflict. An example of this situation would be when females that receive large gifts have higher fitness than females that receive small gifts, while males that provide small gifts have higher fitness than males that provide large gifts. In this scenario, there are differing sex-specific optima for nuptial gift size, which instigates an antagonistic coevolution between the sexes (Gavrilets, 2000; Arnqvist & Rowe, 2005; Fricke, 2009). A predicted by-product of such sexual conflict is that there should be considerable variation among populations in their distribution of gift sizes, as antagonistic evolution is likely to follow different trajectories in different populations (Arnqvist & Rowe, 2005). Moreover, in the extreme circumstance of when males providing no gift have the highest fitness, such sexual conflict could result in the near or complete loss of nuptial gifts. While theoretically possible, evidence for such a loss has not been found previously in nature, even though many studies have documented sexual conflict acting on nuptial gifts (reviewed in Gwynne, 2008).

To evaluate the above predictions about sexual conflict acting on nuptial gift size, we utilized the promiscuously mating *Allonemobius socius* complex of crickets. This group is ideal for such a test as the costs and benefits of gifts appear to be dramatically different between the sexes. Specifically, while males transfer a spermatophore (an ejaculate filled protein capsule) during copulation, the female chews off the tip of a specialized spur on his hind tibia, and cannibalizes his blood (Fedorka & Mousseau, 2002a; Birge et al., 2010). This can represent a large cost for the male as the blood gift has been demonstrated to constitute up to 8% of the male's body mass (Fedorka & Mousseau, 2002a). Additionally, because males mate multiply, the cost of this gift accumulates over matings resulting in a significant reduction in longevity (Birge et al., 2010). Moreover, recent work by DiRenzio & Marshall (2013) suggests that males providing small or large gifts are equally successful at inducing females to lay eggs. Conversely, females have been shown to prefer larger males and gifts (Fedorka & Mousseau, 2002b) and to have longer longevity after consuming multiple gifts (Birge et al. 2010). These sex-specific differences in nuptial feeding can be viewed as an antagonistic coevolution of male and female behaviors – with male behavioral adaptations acting to decrease female feeding time and female behavioral adaptations increasing it. For example, once females mount the male, females may kick at the male's genitalia or stand off center from the male, both of which delay or prevent the male from attaching his spermatophore. On the other hand, males may twitch or kick the hind leg that the female is attempting to feed upon in an effort to decrease female feeding. Additionally, the male may evolve strategies to attach the spermatophore quickly and then immediately break off copulation – once again, reducing or even eliminating nuptial feeding. Therefore, we can think about conflict over the length of nuptial feeding as a set of behavioral adaptations and counter-adaptations that mediate the length of nuptial feeding and thus nuptial gift size.

To determine if nuptial gift size is a target of sexual conflict in this system, we assessed the distribution of gift sizes in twelve populations of the *A. socius* complex. Moreover, we assessed the fitness of males providing different sizes of gift in each population and used the resulting data to generate population-specific fitness profiles – profiles that indicate whether the population is currently evolving towards smaller or larger gifts or something in-between. We also assessed the gift size distribution for three populations at two different time points, spanning up to ten years. This was done to determine if and how gift size profiles are changing over time (i.e., how quickly can such behavioral traits evolve). Lastly, because small gift sizes were common and most populations appear to be evolving towards small gifts, we conducted a detailed behavioral study of the initial stages of gift giving. We found that males providing no gift had higher copulation success than their gift-giving counterparts. In all, our data suggest that sexual conflict is acting on nuptial gift size in this system. Moreover, this antagonistic coevolution can evolve rapidly (over just a few generations) with either sex having the upper hand depending upon the point in the "conflict game" the population is analyzed.

Materials and Methods

Allonemobius socius, *A. fasciatus*, and *A. sp. nov.* Tex adult and juvenile crickets were collected from twelve different locations in the field representing populations present in IL, TX, GA, MO, SC, KS, VA, NJ, and LA. All individuals from the same location were collected concurrently. Crickets were transported to Kansas State University and placed in sex-specific cages, fed *ad libitum* (Purina Cat Chow), kept on a 14:10 photoperiod and bred for one generation (to reduce field effects from our experimental design; Huestis and Marshall 2006a). The new experimental generations were mated 10-15 days post-eclosion to allow time for reproductive maturity.

For individual mating trials, we placed a single virgin female into a plastic container (8x8x4cm) with food and water for 24hrs prior to mating. Then, we removed the food and introduced a single virgin adult male of the same population and allowed them to mate. To instigate copulation, males will court females by song calling and slightly twitching their body near the female (Birge et al. 2010). If the female is receptive, she will mount the male briefly in a mock copulation. Only after this mock copulation will the male produce an external spermatophore. When the spermatophore hardens (~15-20 minutes after it is produced by the male) the male will call to the female again and move his abdomen in front of the female so she can mount him. During this second mount the male attaches the spermatophore to the female while the female chews his specialized tibia to access his hemolymph. Once they separate, it is possible to determine if the copulation was successful (i.e., the spermatophore is attached to the female) or not (i.e., the spermatophore is not attached to the female). If successful, the spermatophore will remain attached to the female until she wipes it off (at which point she usually eats it as well). After a successful copulation, females were isolated in egg-laying cages and placed in environmentally controlled incubators at 29C. Cheesecloth was provided as an oviposition substrate for females. Females were given four days to lay eggs, after which we counted the number she laid.

For each trial, we measured the duration of second mount (i.e., copulation time), which was used as a proxy for nuptial feeding time (i.e., gift size), as these two measures are correlated (Fedorka & Mousseau, 2002a). While measuring the duration of second mount or the length of time a female feeds on a male during copulation is relatively straight forward, it is important to remember that this measure is a composite phenotype – i.e., a phenotype that is made up of multiple phenotypes. In this case, gift size is a composite of multiple behavioral phenotypes, including male spermatophore attachment behavior, male kicking behavior, female mounting behavior (i.e., correctly positioned or not), female kicking at the male genitalia behavior, female chewing behavior, and both male and female movement behaviors. All of these behaviors are centered around gaining or preventing access to the male's specialized tibial spur from which the female can feed on the male's hemolymph.

We also measured spermatophore attachment time (sperm transfer) and the number of eggs laid (a measure of fitness). Although collection and mating trials for the populations took place periodically between 2000 and 2013, each population's matings and treatments were held constant (e.g., females were only allowed to mate once and females given a set number of days to lay eggs). A phylogeny was generated for the populations collected using published data (Howard & Furth, 1986; Marshall, 2004; Huestis and Marshall 2006b) and the knowledge that isolation-by-distance occurs among populations within a species. The outgroups with nuptial feeding are *Pictonemobius* (Gross et al. 1989) and *Eunemobius* (Piascik et al., 2010), while the outgroups without nuptial feeding are *Hygronemobius* and *Nemobius* (Mays 1971). These papers show that the specialized tibial spur and the associated nuptial feeding is limited to a subgroup of ground crickets. This phylogeny was used to show the distribution of gift sizes across the phylogeny and analyze evolutionary patterns in the change in gift size over time (see Chapter 3).

Fitness was estimated by calculating the relative number of eggs a female laid for each of the twelve populations. For each population, the female laying the most eggs was given a fitness score of 1 (this number of eggs laid was considered the maximum number of eggs a female could possibly lay in that particular population). For all other individuals in that population, their fitness was calculated as “their number of eggs laid” divided by “the maximum number of eggs laid” from above. This resulted in fitness ranging from 0 to 1 in all populations, thus allowing comparisons to be made across populations.

To generate the gift size distribution for each population, we used histograms to categorize gift size frequency into 2-minute intervals (ranging between 0 and 32 minutes which captured >99% of all values). For each gift size interval, we calculated the average fitness and then used the resulting data to construct a fitness distribution relative to gift size. For each population, we calculated a correlation coefficient (r) to determine if there was a significant relationship between average fitness and gift size.

Next, for each population, we generated a population-level fitness profile to determine the direction, if any, that selection is currently driving the gift size phenotype. To do this, we multiplied the actual number of occurrences (frequency) in each gift size category by the relative fitness of that gift size category. This yields a “total fitness” value for each gift size category.

We then used linear and non-linear regression models to determine the curve that best described the relationship between total fitness and gift size.

Given that many of the populations are evolving towards small gifts (i.e., feeding times ranging between 0-2 min), we needed to assess the probability that females could gain access to male hemolymph during a mating. To test this, we designed an experiment that would allow us to unequivocally determine whether or not a blood gift was available to females (gift vs. no gift) at different times based on the condition of the male's hind tibia after a mounting. To begin, a virgin male was introduced into a female's cage (experimental protocols as above) and was allowed to copulate for one of three experimental times (0.5, 1, and 2 minutes); time was started after the female had settled into her mounted position. When the experimental time had elapsed, we separated them by lightly disturbing the mating cage; the males were then immediately put into deep freeze (-80°C). We recorded how long the female actively chewed on the male tibia during her mount as well as if the male was successful in transferring his spermatophore. Each mated pair was used once and at least 10 trials were conducted for each of the three time periods. After the trials were completed the males were thawed and the specialized tibia was visually inspected under a dissecting microscope to look for feeding damage. To confirm that a female had or had not gained access to the male's hemolymph, we then lightly squeezed the femur with forceps forcing the hemolymph distally though the leg. If a female had gift access, the hemolymph would flow out of the tibial spur wound; if the female did not have gift access the hemolymph would not flow out. The tibial wound will clot over time but this was dismissed as a distorting factor as we performed the squeeze test within 1 minute of thawing (typical clot times were always greater than 5 minutes).

Results

The gift size distributions, fitness distributions, and population profiles for the twelve populations collected are summarized in a phylogeny in figure 2.1. The majority of the populations had gift size distributions that show smaller gifts as being the most abundant; in fact, many of these populations have the smallest gift size possible as the most frequent (i.e., populations a, c, d, e, f, k, l). Two populations have intermediate values for both large and small gifts (g, i) and one population showed a high frequency of large gifts (j). In assessing the fitness distributions of various gift sizes for each population, we found that relative fitness did not significantly correlate with either small or large gifts for most populations: [(a) r=0.26, N= 24, *P*=0.57; (b) r=0.07, N=38, *P*=0.85; (c) r=0.45, N=11, *P*=0.31; (d) r=0.39, N=19, *P*=0.34; (e) r=0.36, N=9, *P*=0.54; (f) r=0.10, N=38, *P*=0.77; (g) r=0.31, N=15, *P*=0.38; (h) r=0.31, N=34, *P*=0.49; (i) r=0.30, N=12, *P*=0.51; (j) r=0.22, N=12, *P*=0.60; (k) r=0.34, N=16, *P*=0.41; (l) r=.83, N=20, *P*=0.17]. We combined fitness and gift size frequency data to create population profiles of total fitness to establish the evolutionary tendency of each population. Our results indicate that in the majority of populations there likely is strong (i.e., see populations a, d, and l) or moderate/weak (i.e., populations b, c, e, f, and k) selection for the reduction or loss of gift size as evidenced by the negative-exponential or negative-linear “total fitness” profile curves, respectively (Figure 2.1). On the other hand, two populations showed “total fitness” profiles with near zero slope (i.e., populations g and h), while two others show a slight positive, linear slope indicative of weak selection for larger gift sizes (i.e., populations i and j). Together, it is clear there is variation among populations and that selection is acting differently in different populations.

For three of the populations we compared gift size distributions and total fitness profiles between two time points that were several years apart (Figure 2.2). The Georgia population data from 2008 indicates a slight negative slope in the total-fitness profile (as while small gift sizes are more common, fitness is higher with larger gifts). However, in five years (i.e., by 2013), the median gift size shifted from 6 to 7 minutes (for a rate of change of 0.1 minutes/generation as populations in Georgia go through two generations per year). However, paradoxically, there was a significant shift in the total-fitness profile towards small gift sizes (Figure 2.2a). In all, it appears that a changing fitness profile may explain these results, but more data are needed.

Results from the other two populations (i.e., Illinois and New Jersey) are clearer. For example, the Illinois population shifted from a near even distribution with a total-fitness profile showing a slope of zero (or bimodal depending on the curve) in 2009 to a small-gift biased population with a significant negative slope in the total-fitness profile in 2013 (Figure 2.2b). The median gift size shifted from 6.5 to 4 minutes in 4 years, resulting in a 0.625 minute/generation rate of change. The New Jersey population yielded the most dramatic results, as the gift size distribution not only shifted from being evenly distributed in the year 2000 to small-gift dominant in 2010, including a change in median gift size from 12 minutes to 1 minute during those 10 years (Figure 2.2c). This corresponds to a 1.1 minutes/generation rate of change. To put that into perspective, a population could evolve from a median gift size of 32 minutes (which are very large gifts similar to the outgroups) to a median gift size of 0 minutes (i.e., no gift) in 29 generations – or as few as 14 years for populations that go through two generations per year.

The results of the tibial feeding experiment (Figure 2.3) suggest that the probability of the female receiving a gift (hemolymph access) is low for feeding times less than 2 minutes. For feeding times of 30 seconds and 1 minute, the probability of gift access was 0.28 and 0.25, respectively. At 2 minutes, the probability increases to 0.70. It is clear that when the female has brief access to the tibial spur she is unlikely to consume male hemolymph. We also assessed the probability of the male attaching his spermatophore (i.e., a successful copulation) during each of the three time points. We found that males were able to attach their spermatophore regardless of whether or not the female fed on hemolymph. In fact, the probability of a successful mating was higher for males that did not provide a gift compared with those that did for all three time points – i.e., if a male did not provide a gift to the female during copulation it was more likely that he would successfully attach his spermatophore.

Discussion

From our results, nine of the populations appear to be under selective pressures to reduce gift size, two populations under selection to increase gift size and one exhibits no signs of the direction of selection. We infer that variation in nuptial feeding in the *A. socius* complex is the consequence of sexual conflict rather than sexual selection. If sexual selection was acting on gift size, we would expect to see gift size settle at some large-gift size equilibrium in which sexual selection is balanced by natural selection (thus generating only relatively minimal variation among populations). Instead, our data indicates significant variation among populations with apparent selection for small gift sizes in some and some large gift sizes in others – a predicted consequence of sexual conflict.

In the *Allonemobius socius* complex, antagonistic coevolution (conflict over outcomes of interactions) between males and females appear to be the result of diverging evolutionary interests for optimality of gift size. Sexual conflict under this type of scenario is context dependent as different populations will have varying genotypic constitutions and environmental compositions. Because of this, one would expect variation in how sexually antagonistic coevolution is acting on populations depending on which sex has the upper hand in the arms race.   The direction and intensity of how the pendulum of antagonistic selection and coevolution swings can be viewed as a function of the ratio between costs and benefits of providing or consuming a gift, individual condition and environmental contexts. Furthermore, the promiscuous nature of males and females in the *Allonemobius socius* complex emphasizes the potential for sexual conflict to act aggressively as compared with monogamous species which show a reduction in the extent of sexual conflict (Chapman, et al., 2003; Arnqvist & Rowe, 2005).

The evolutionary shifts in nuptial feeding (i.e., gift size) observed from this study implicate sexual conflict as its selective force, but what are the specific phenotypic adaptations that are acting to generate this conflict? The conflicting adaptations in *Allonemobius* appear to be rooted in the evolution of the male tibial spur, which presumably initially evolved by sexual selection in which both males and females gained a fitness benefit. In fact, it is difficult to envision this feature initially evolving if it did not represent a fitness benefit to both sexes. Females benefited from an 'honest' gift while males could distract the females long enough to attach his spermatophore. Over time though, males and females evolved new behavioral adaptations and counter-adaptations due to the presence of the tibial spur. Such novel behaviors that arise and evolve around a new morphological feature are commonly found in nature (West-Eberhard, 2003). The tibial spur is potentially highly exploitative by the female (i.e., taking too large of a gift) and it is the behaviors of the sexes in response to the easily exploitive nature of the tibial spur that appear to be generating the observed conflict.

Given gift size is a composite phenotype, it is important to consider what male and female behaviors are important in this conflict and what their consequences may be on the evolution of this phenotype. For example, female behaviors can be displayed through her propensity to consume as much hemolymph as possible, whereas males may display a kicking behavior that deters or at least delays the female chewing. It is not hard to image how the behavior of kicking by the male could have evolved in response to the lowered fitness he incurs due to the female's over consumption of his hemolymph. It is the female who has control when she chooses to mount a male, but both sexes can manipulate the duration of feeding and copulation. Ultimately, it is selection on these specific behaviors that is the mediator of sexual conflict. Given that this conflict is ongoing and that different populations are likely following different evolutionary trajectories, we predict that the sex with the 'upper hand' will vary among populations. In some cases, females may have the upper hand and thus they should exhibit behaviors (e.g., mounting position, resistance to copulation termination, and kicking at the males genitalia during copulation) that enhance their control over the length of feeding time – yielding longer nuptial feeding times and larger gift sizes. This would place strong selection pressure on males to evolve counter adaptations. However, if males have the upper hand, then we would expect to see male behaviors (e.g., moving his leg to interfere with female feeding and rapid spermatophore attachment) that significantly decrease or eliminate nuptial feeding. Which, once again, would place selection pressures back on females to counter these male behavioral adaptations.

An integral component to these conflicting behaviors is their effect on the cost/benefit economy of the mating system depending on which sex has the upper hand. The costs of feeding for the male are high and is limited by his body size as the tibial spur from which the hemolymph gift exudes is not a compartmentalized and separate structure but is a channel to the whole of his hemolymph (Fedorka & Mousseau, 2002a). Not only is there a cost in hemolymph loss (lifespan) but it also opens them up to detrimental parasitic invaders. In fact, there is sexual dimorphism in the immune system, with males carrying a larger hemocyte load and lytic activity than females; males have to immunologically prepare themselves for the costs of mating and feeding (Fedorka et al., 2004). Because of this, one would expect males to minimize feeding as much as possible through behavioral adaptations. On the other hand, some females have evolved to discriminate against small gift giving males. One experiment was designed to see the effects of denying hemolymph access by covering the male tibial spur with a paint that could not be chewed through; females that encountered males with a covered tibia were more likely to terminate copulation much earlier than controls even though nutritional resources are not limited in natural populations or in lab experiments (Bidochka & Snedden, 1985). This particular experiment in comparison to our data shows the dynamism of sexual conflict; in particular, it shows females may use the early termination of a copulation as a strategy to limit a male's success while in many of the populations we studied males used the quickly attach your spermatophore and terminate copulation strategy. In all, it is no surprise that nuptial feeding in this system is ripe with the potential for sexual conflict to unfold along any point of the conflict continuum. Our evidence suggests that in many of the populations, the costs of feeding incurred by the males are becoming so high that these populations are evolving towards the loss of this trait – as a by-product of male behavioral adaptations.

Interestingly, our data not only suggest that sexual conflict is driving the evolution of gift size, but that this evolution is occurring rapidly over just a few generations. It may be surprising that conflict is selecting this rapidly for the reduction of gift sizes, but theory predicts the rapid evolution of traits related to sexuality (Fisher, 1930; Lande, 1981). This is further reinforced by the fact that nuptial feeding is a composite behavior phenotype. Behavioral traits can range from being extremely labile and highly plastic to relatively static (innate behaviors). While behavior has often been thought of as more responsive to the environment than morphology, there has yet to be a consensus on the rates, mechanisms, and pathways of behavioral evolution as compared with morphology (West-Eberhard, 2003; Duckworth, 2008).

Our study provides a glimpse of the magnitude and direction in which sexual conflict is acting on *Allonemobius socius* complex populations. There are several questions remaining though. Given that most of the populations currently appear be evolving toward the reduction of gift size, would we expect the complete loss of nuptial feeding in any of these populations? Under any selection criteria one would expect the most abundant traits to be the ones that are going to perpetuate themselves. If it is common that males successfully transfer their genetic material and then terminate copulation without the cost incurred from feeding they can mate more frequently and increase the presence of the non-feeding phenotype. A possible outcome of this is the complete loss of nuptial gifts for these populations. Furthermore, our results beg further research into the dynamics of conflict, as our study was conducted under uniform environmental conditions. It is important when studying conflict to expose the study organisms to a range of ecological settings as this can have profound impacts on the intensity and economics of conflict. Fricke (2009) sites a study where male flies harass females more aggressively when the mating arena contained the natural substrate on which the female normally oviposits. Conducting studies that include environmental factors, especially factors related to the promiscuous nature of females (i.e., the effects of multiple matings) relative to conflict, would be an important next step to address the possibility of plasticity of conflict in this system. For example, is the strength of conflict mediated by density of individuals? Are males more likely to be fed upon longer if there are potential rivals present? Resolving such questions is necessary to gain a full picture of the dynamics of the strength conflict.

Figures

Figure .2.1 Phylogeny of Gift Sizes

Phylogeny of gift size distributions, fitness distributions, and population profiles in the *Allonemobius* complex: (a) South Carolina 1 (b) South Carolina 2 (c) Georgia (d) New Jersey (e) Petersburg, Virginia (f) Manhattan, Kansas (g) Fenton, Missouri (h) Illinois (i) Louisiana (j) Texas 1 (k) Texas 2 (l) Frankfurt, Illinois (m) *Pictonemobius* (Gross et al., 1989) (n) *Eunemobius* (Piascik et al., 2010) (o) *Hygronemobius* and other Nemobiinae (Mays, 1971).

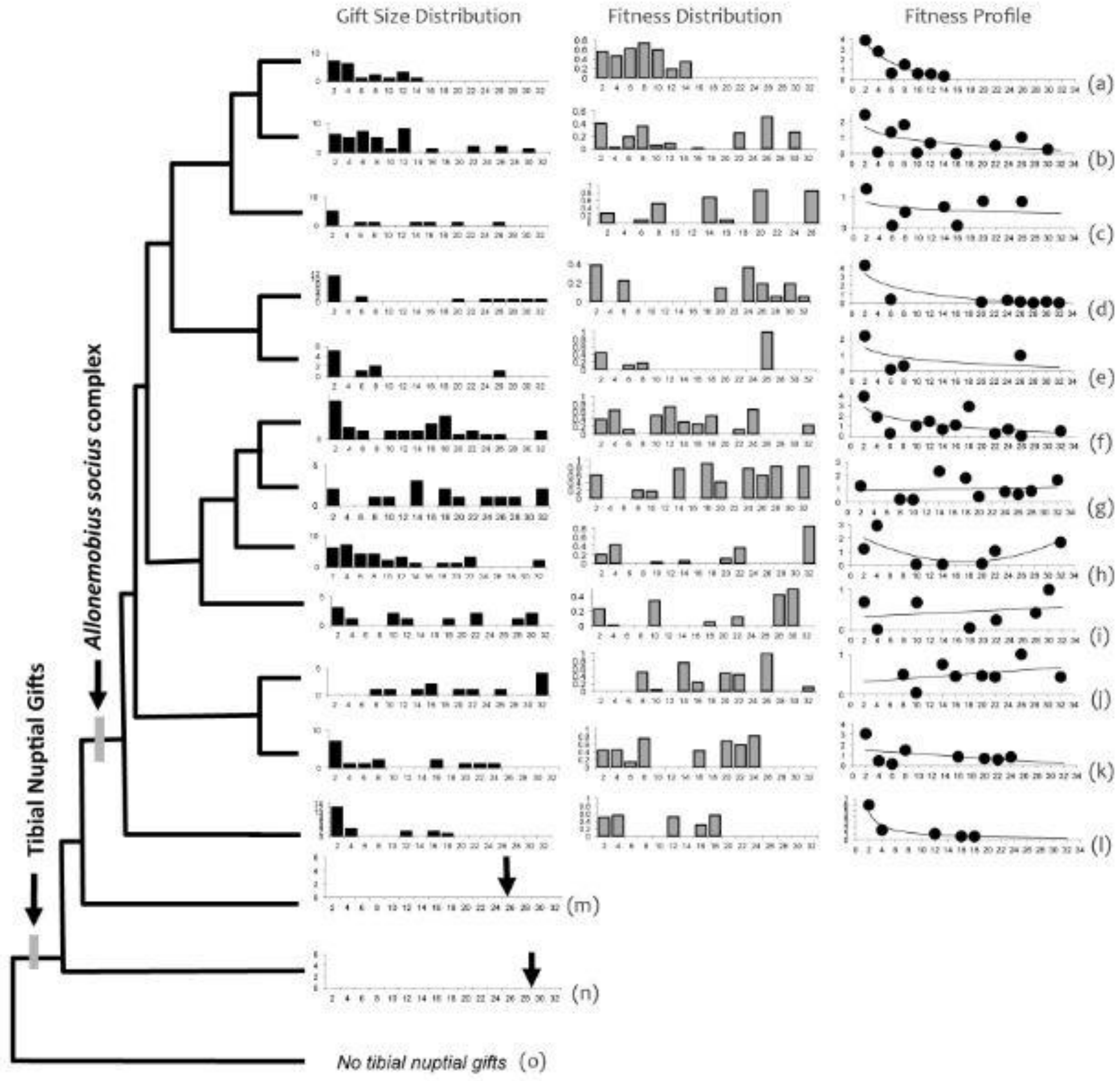


Figure 2.2 Population Profiles

Population profiles (a, b), and gift size distributions (c) for two different time points for three populations of *A. scoius*.

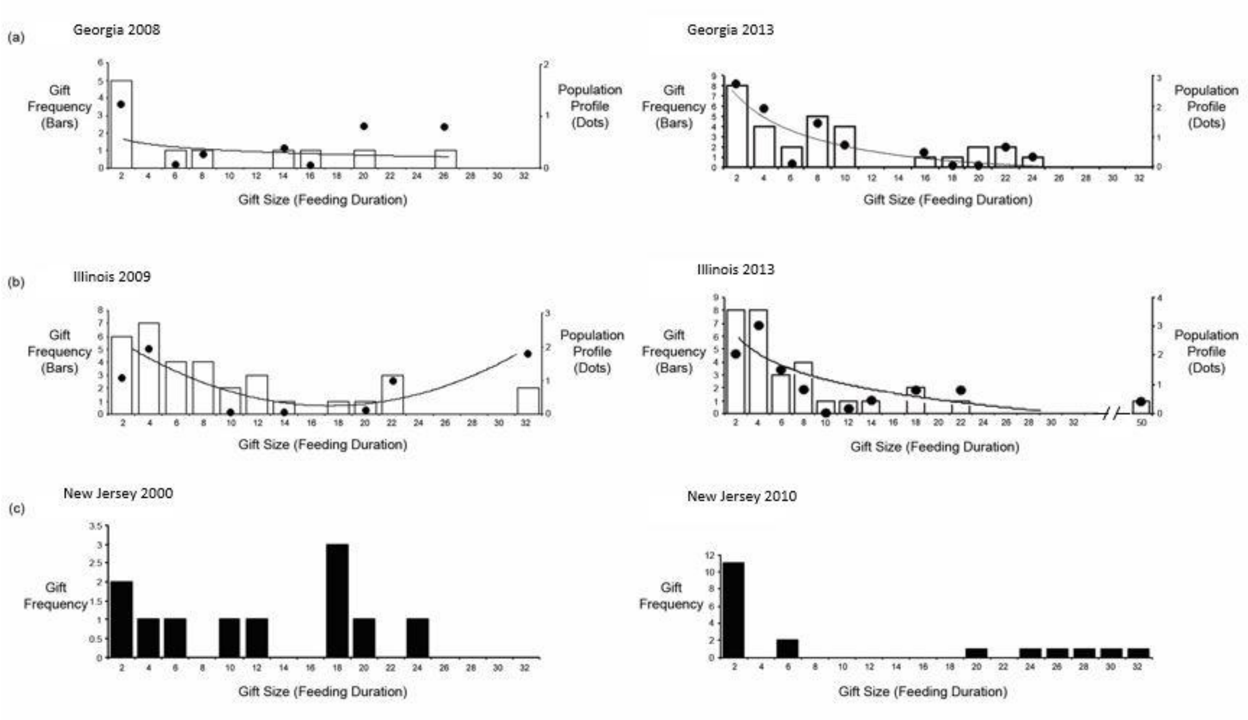
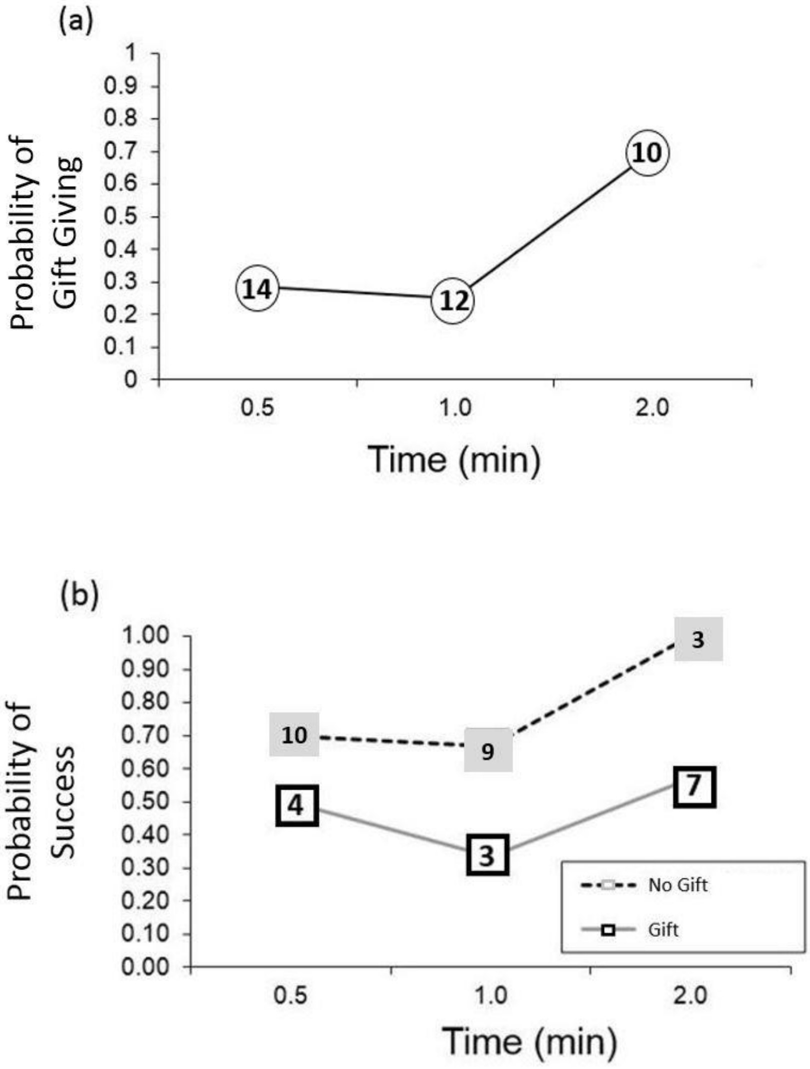


Figure 2.3Tibial Feeding

Tibial Feeding. (a) Probabilities of giving a gift and (b) probability of successfully attaching the spermatophore whether giving a gift or not.



Chapter 3 - Pursuing Unanswered Questions

Filling in Gaps Using the Comparative Approach

One of the most ambiguous and under researched aspect of sexual conflict is how intensely conflict acts to drive differences in the outcomes of interactions between males and females. The most compelling evidence comes from a study in *Drosophila* in which females were experimentally arrested so they could not evolve counter adaptations to male mating persistence; it was found that within 30 generations males could radically and harmfully circumvent the initial female resistance traits (Rice, 1996). While this demonstrates that conflict can act aggressively in a short amount of time, it does not address the inherent property of conflict - that the strength of antagonistic selection fluctuates based on the current adaptational ability of a particular sex at a particular time. It is difficult to assess the strength of conflict looking at only one population at one point in time, as there is no way to assess the relative effect of conflict. A population may be undergoing intense sexually antagonistic coevolution, but because it may happen to be in a state in which male and female counter adaptations are balanced, the outcome of the interaction appears to be non-conflicting. The only way to resolve this is to look at different populations and different points in time.

Dawkins (1976) envisioned sexually antagonistic coevolution as a pendulum that swings back and forth depending on the level of adaptation for a particular sex. A static hanging pendulum indicates a fluctuation away from the extremes of an interaction (neither males nor females have the upper hand), while any deviation from that predicts a male or female advantage concerning mating. No matter what the position, it is expected that the pendulum is always moving due to adaptations and counter-adaptations between the sexes. Typically, we are only able to get a snapshot of one moment in time of the position of that pendulum. Comparative approaches, like my results, suggest not only the direction in which the pendulum is swinging (based on different time points) but a potential range in which it can swing (comparing across populations). If we imagine the two extremes when the pendulum is exactly horizontal (0˚ and 180˚), we may say that males or females have completely circumvented the opposition's persistence/resistance. The reality though is that the range of the pendulum will be somewhere in between. The question then becomes how far can the pendulum swing?

By using a comparative approach, my research is the first to show an adaptational landscape in which inferences can be made about the pressure that sexual conflict exerts on natural populations. The theoretical extremes would be that either the male has 'won' and is able to mate successfully without providing a nuptial gift or that the female has 'won' and feeds until satiation (this should be impossible due to counter-adaptations). Instead, limits are observed that show that both males and females can have the upper hand in the outcome of mating, but that one sex does not have a monopoly on 'winning' as winners and losers are transient states. By looking at the data it is easy to say which sex has the upper hand at a particular time, by looking at the position of the pendulum (i.e., the angular amplitude, Θ). But, we can also assess the relative strength of conflict between populations by analyzing the slope of each of the population's profiles (comparing the position of pendulum swings from one population to another). In our case, a negative, linear slope indicates that males have the upper hand but a negative exponential trend indicates that conflict is acting more intensely – thus, the selection acting on males through sexual conflict is stronger. Again, this only represents one time point and any of these populations could sway to either end of the spectrum over time depending on the interplay of adaptations and counter-adaptations.

For the first time, we can assess the dynamics of conflict and assess its boundaries for a group of closely related populations. Never before has the malleability of sexual conflict been explicitly demonstrated; therefore, this study adds an important data point to the biology of conflict. Furthermore, I was able to demonstrate the rates at which antagonistic coevolution can occur over time by comparing two time points in three populations (Figure 2.2) by calculating the ratio of median gift size and number of generations. For example, the Georgia population decreased its tendency for large gift sizes by 0.1 minutes/generation and the Illinois increased its tendency by 0.625 minutes/generation. The most dramatic change occurs in the New Jersey population in which what appeared to be an equilibrium state between males and females over nuptial gift size, turned out to be a transient state where males and females were in flux in an adaptational arms race; a moment in which the pendulum was at its equilibrium point, yet its velocity (strength of conflict) was maximized and positioned to swing in one direction or the other. Within ten years, male's counter adaptations were more effective and resulted in a tendency for shorter feeding times in that population populations by 1.1 minutes per generation, a significant change in just a handful of generations indicating that sexual conflict was strong and acts quickly, an observation consistent with Rice's (1996) experiment. A major limit to studying conflict is that it can be hidden, that an intense sexually antagonistic arms race can be occurring but because of its particular state, is unobservable (Arnqvist & Rowe, 2005). The only way to assess direction of conflict is to have more than one data point so comparisons can be made. My results show that sexual conflict hidden by transience can be exposed by tracking populations over time.

Finally, the comparative approach can be used to infer phylogenetic histories based on gift size distributions using median gift sizes to establish ancestral states (Figure 3.1).

Interestingly, gift size divergence is correlated with species branching events between the three species in the *Allonemobius* complex. For example, the bifurcation between *A. fasciatus* and *A. sp. nov.* Tex show completely different trajectories for gift size, both a significant decrease and increase, respectively. It is clear that speciation and sexual conflict are somehow intertwined. Either sexual conflict instigated speciation at these divergence points or sexual conflict became exaggerated shortly after speciation events which has been demonstrated before (Gavrilets, 2000). Which of the processes is the causative factor of the other remains unclear. Furthermore, we see a dramatic fluctuation in gift sizes happening in more derived populations. In the Texas populations (TX1 and TX2), we see two extreme states of gift size divergence. Even though these populations presumably share a very recent common ancestor, in the short time since divergence there has been significant selection pressures differentiating the two populations in terms of gift size. Rapid selection between extremes is also seen within short amounts of time as seen in the NJ1 population which indicates just how quickly conflict can generate differing evolutionary trends. In this population, a rapid swing toward small gift sizes are occurring in the extant species studied. Interestingly, the ancestral estimation shows the opposite trend with larger gift sizes being selected over time; this population demonstrates the most extreme variation in gift size tendencies. It appears, even only looking at the tips of the phylogeny, that the pendulum of conflict is swinging wide and quickly over short periods of time giving an indication of the magnitude in which sexual conflict acts on populations.

Future Directions

It is a long held assertion that monogamous species are less likely to exhibit sexual conflict compared to promiscuous species, as the opportunity for males to develop unique strategies that prevent other males from mating with the same female are repressed. Rice (1996) demonstrated how fast harmful phenotypes in males can evolve when female evolution is arrested. But what are the predictions to be made when a population experiencing intense conflict suddenly eliminates the basis for that conflict by arresting promiscuity? *Allonemobius* is one particularly good complex in which to answer this question because it is, as shown above, in the midst of sexually antagonistic coevolution and they are highly promiscuous.

Taking a population that exhibits high levels of conflict (gift size extremes) and forcing monogamy in the lab could answer unresolved questions about sexual conflict. The expectation would be that over generations the population profiles would exhibit a suppression in the level of conflict, the slope of the profile would move from negative exponential to negative linear to near zero. The question then becomes how many generations would it take to see a significant reduction in the phenotype. While this is a lab experiment and is not indicative of what would be happening in natural populations, it does indicate the dynamism of sexual conflict and could glean information about the genetic basis of sexual conflict (how many genes are involved). If a population only takes a few generations to suppress conflict it may be surmised that the genetic basis of the phenotype is much less complex compared with a population in which it takes thousands of generations for conflict to be suppressed. Ultimately, this approach could inform just how strong the hold of sexual conflict is over populations.

Another question that could be answered from forced monogamy over time is how population fitness is affected by conflict. Theoretically and empirically, sexual conflict has been demonstrated to reduce population fitness in promiscuous species (Arnqvist & Rowe, 2005). How would population fitness change when a population goes from a highly promiscuous and highly conflicting population to one that has been experimentally forced into monogamy over many generations? There are several aspects to consider. First, there are the reproductive load fitness costs from conflict; besides the phenotype of interest, other reproductive phenotypes may be playing a part in reducing population fitness. For example, the ejaculate of males in *Drosophila* have seminal fluid proteins that act to stimulate egg laying and to increase the refractory period for females to re-mate (Rice, 2000). There are also adaptational loads to consider. When a sex evolves counter adaptations to a particular stress, the genetic response may not be a direct answer to the pressure exerted as the genetic basis of responses to conflict are presumably complex. Adaptive load artifacts that accumulate because of the see-sawing of conflict could affect fitness negatively. While it would not be possible to parse out all of the effects, this approach does have the ability to assess the all-encompassing effect conflict has on population fitness.

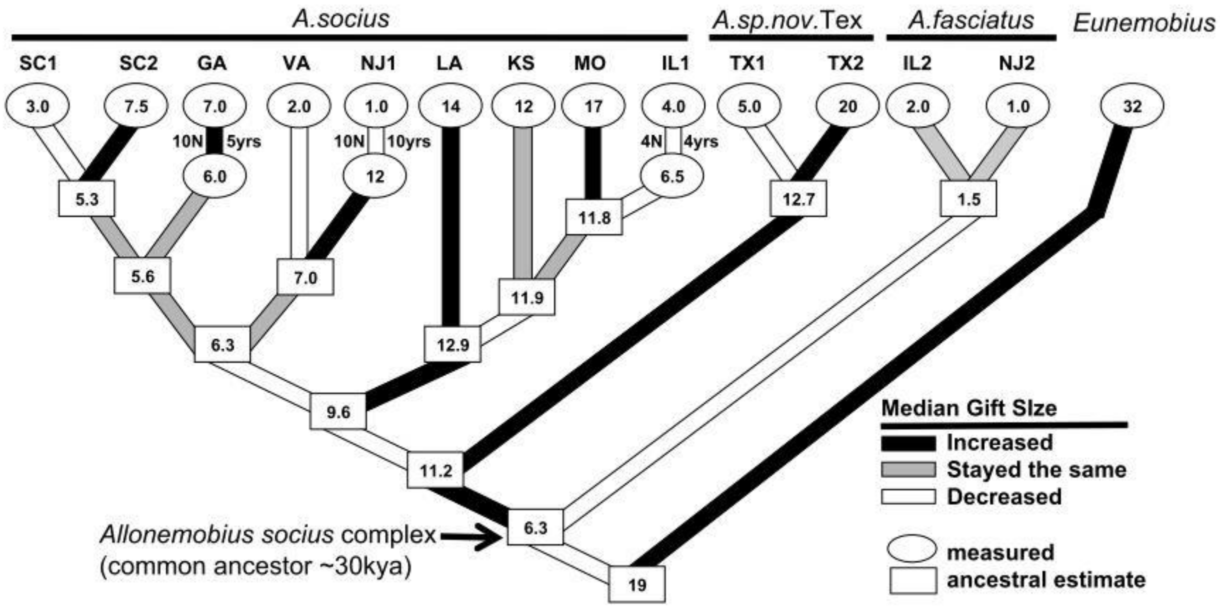
Finally, an important avenue of continued research would be to expand upon the comparative approach outlined above. The strength of the comparative method in relation to sexual conflict is that levels of conflict can be compared across taxa. While simply comparing the same phenotype conflict is acting on between more diverging taxa would be informative, this approach can potentially be taken further. Instead of using divergence as a comparative basis, can biological and behavioral traits be compared in light of conflict? One biological metric would be the level of promiscuity across species. Promiscuous species range in how often they mate, from low levels to high. Using population profiles, we can assess how strong conflict is acting on a range of promiscuous species, with promiscuity being the only criteria for comparing organisms. The caveat to this is the phenotype conflict is acting on would have to be the same across species. If one used a common conflict phenotype like mating rate, would we see a correlation between level of promiscuity and level of conflict? While it may seem intuitive that would be the case, it has yet to be explicitly demonstrated.

Even though sexual conflict has been recognized since Darwin, there remains a surplus of unanswered questions. Much of conflict research stems from the theoretical realm and there remains many tests to assert the validity of these theoretical predictions. It is clear that conflict has played an immense role in shaping the biology of organisms and there is little doubt that pursuing investigative research concerning the power of sexual conflict will continue to answer essential questions in biology.

Figures

Figure 3.1 Historical Phylogeny

Phylogeny showing median gift size of extant populations and mapping ancestral state estimates of gift size.



References

Aiken, RB. 1992. The mating behavior of a boreal water beetle, *Dytiscus alaskanus* (Coleoptera: Dytiscidae). Ethology Ecology & Evolution. 4: 245-254.

Aiken, RB & A Khan. 1992. The adhesive strength of the palettes of males of a boreal water beetle, *Dytiscus alaskanus* (Coleoptera: Dytiscidae). Canadian Journal of Zoology. 70: 1321-1324.

Arnqvist, G. 2004. Sexual conflict and sexual selection: lost in the chase. Evolution. 58: 1383-1388.

Arnqvist, G, M Edvardsson, U Friberg, T Nilsson. 2000. Sexual conflict promotes speciation in insects. PNAS. 97: 10460-10464.

Arnqvist, G & L Rowe. 2002. Correlated evolution of male and female morphologies in water striders. Evolution. 56: 936-947.

Arnqvist, G & L Rowe. 2005. Sexual Conflict. Princeton University Press, Princeton, New Jersey.

Bastock, M. 1956. A gene mutation which changes a behavior pattern. Evolution. 10: 421-439.

Bidochka, MJ & WA Snedden. 1985. Effect of nuptial feeding on the mating behaviour of female ground crickets. Canadian Journal of Zoology. 63: 207-208.

Birge, LM, AL Hughs, JL Marshall, DJ Howard. 2010. Mating behavior differences and the cost of mating in *Allonemobius fasciatus* and *A. socius*. Journal of Insect Behavior. 23: 268-289.

Boggs, CL. 1995. Male nuptial gifts: phenotypic consequences and evolutionary implications. Insect Reproduction (eds. SR Leather & J Hardie) CRC Press: 215-242.

Bulmer, MG & GA Parker. 2002. The evolution of anisogamy: A game-theoretic approach. Proceedings of the Royal Society of London Series B. 269: 2603.

Carayon, J. 1966. Tramatic insemination and paragenital system. In: Monograph of Cimicidae. Entomological Society of America, College Park, MD.

Cameron, E, T Day, L Rowe. Sexual conflict and indirect benefits. Journal of Evolutionary Biology. 16: 1055-1060.

Chapman, T, G Arnqvist, J Bangham, L Rowe. 2003. Sexual Conflict. Trends in Ecology and Evolution. 18: 41-47.

Chapman, T & SJ Davies. 2004. Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies. Peptides. 25: 1477-1490.

Chapman, T. 2001. Seminal fluid-mediated fitness traits in *Drosophila*. Heredity. 87: 511-521.

Chen, PS. 1996. The accessory gland proteins in male *Drosophila*: Structural, reproductive, and evolutionary aspects. Experientia. 52: 503-510.

Cordero, C & WG Eberhard. Female choice of sexually antagonistic male adaptations: a critical review of some current research. Journal of Evolutionary Biology. 16: 1-6.

Dawkins, R. 1976. The Selfish Gene. Oxford University Press, Oxford.

DiRienzo, N & JL Marshall. 2013. Function of the hemolymph nuptial gift in the ground cricket, *Allonemobius socius*. Ethology. 119: 104-109.

Duckworth, RA. 2008. The role of behavior in evolution: a search for mechanism. Evolutionary Ecology. 23: 513-531.

Eggert, AK & SK Sakaluk. 1994. Sexual cannibalism and its relation to male mating success in sagebrush crickets, *Cyphoderris strepitans* (Haglidae: Orthoptera). Animal Behaviour. 47: 1171-1177.

Fedorka, K, M Zuk, T Mousseau. 2004. Immune suppression and the cost of reproduction in the ground cricket, *Allonemobius socius*. Evolution. 58: 2478-2485.

Fedorka & T Mousseau. 2002a. Tibial Spur feeding in ground crickets: Larger males contribute larger gifts. Florida Entomologist. 317-323.

Fedorka & T Mousseau. 2002b. Nuptial gifts and the evolution of male body size. Evolution. 56: 590-596.

Fisher, RA. 1930. The Genetical Theory of Natural Selection. Clarendon Press. Oxford.

Fricke, C. 2009. The conditional economics of sexual conflict. Biology Letters. 5: 671-674.

Gavrilets, S & D Waxman. 2002. Sympatric speciation by sexual conflict. PNAS. 99: 10533-10538.

Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. Nature. 403: 886-889.

Getty, T. 1999. Chase-away sexual selection as noisy reliable signaling. Evolution. 53: 299-302.

Goodly, J. 1976. Production and Reproduction: A Comparative Study of the Domestic Domain. Cambridge: Cambridge University Press.

Gross, SW, DL Mays, TJ Walker. 1989. Systematics of *Pictonemobius* ground crickets (Orthoptera: Gryllidae). Transactions of the American Entomological Society. 115: 433-456.

Hiraiwa-Hasegawa, M. Adaptive significance of infanticide in primates. Trends in Ecology and Evolution. 3: 102-105.

Howard, DJ & DG Furth. 1986. Review of the *Allonemobius fasciatus* (Orthoptera: Gryllidae) complex with the description of two new species separated by electrophoresis, songs, and morphometrics. Annals of the Entomological Society of America. 79: 472-481.

Hrdy, SB. 1979. Infanticide among animals: A review, classification, and examination of the implications for the reproductive strategies of females. Ethology and Sociobiology. 1: 13-40.

Huestis, DL & JL Marshall. 2006a. Is natural selection a plausible explanation for the distribution of *Idh*-1 alleles in the cricket *Allonemobius socius*? Ecological Entomology. 31: 91-98.

Huestis, DL & JL Marshall. 2006b. Interaction between maternal effects and temperature affects diapause occurrence in the cricket *Allonemobius socius*. Oecologia. 146: 513-520.

Kessel, EL. 1955. The mating activities of balloon flies. Systematics Zoology. 4: 97-105.

Kruger, DJ. 2008. Young adults attempt exchanges in reproductively relevant currencies. Evolutionary Psychology. 6: 204-212.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. PNAS. 78: 3721-3725.

Lawrence, SE. 1992. Sexual cannibalism in the praying mantid, *Mantis religiosa*: a field study. Animal Behaviour. 43: 569-583.

Lehmann, G. 2012. Weighing costs and benefits of mating in bushcrickets (Insecta: Orthoptera: Tettigoniidae), with an emphasis on nuptial gifts, protandry and mate density. Frontiers in Zoology. 9: 19.

Maire, N & M Doebeli. 2001. Evolutionary branching and the evolution of anisogamy. Selection 2. 1-2: 119-131.

Marshall, JL. 2004. The *Allonemobius*-Wolbachia host-endosymbiont system: evidence for rapid speciation and against reproductive isolation driven by cytoplasmic incompatibility. Evolution. 58: 2409-2425.

Maynard Smith, J. 1974. The theory of games and the evolution of animal conflicts. Journal of Theoretical Biology. 47: 319-335.

Mays, DL. 1971. Mating behavior of Nemobiine crickets – *Hygronemobius*, *Nemobius* and *Pteronemobius* (Orthoptera: Gryllidae). Florida Entomologist. 54: 113-126.

Parker, GA. 1979. Sexual selection and sexual conflict. In: Sexual selection and reproductive competition in insects. Academic Press, London.

Piascik, EK, KA Judge, DT Gwynne. 2010. Polyandry and tibial spur chewing in the Carolina ground cricket (*Eunemobius carolinus*). Canadian Journal of Zoology. 88: 988-994.

Pigliucci, M. 2001. Phenotypic Plasticity: Beyond Nature and Nurture. Johns Hopkins University Press. Baltimore.

Pizzari, T & RR Snook. 2003. Sexual conflict and sexual selection: chasing away paradigm shifts. Evolution. 57: 1223-1236.

Pizzari, T. 2003. Food, vigilance, and sperm: the role of male direct benefits in the evolution of female preference in a polygamous bird. Behavioural Ecology. 14: 593-601.

Reinhardt, K, R Naylor, MT Siva-Jothy. 2003. Reducing a cost of traumatic insemination: Female bedbugs evolve a unique organ. Proceedings of the Royal Society of London Series B. 270: 2371-2375.

Rice, WR. 1996. Evolution of the Y sex chromosome in animals. Bioscience. 46: 331-343.

Rice, WR. 2000. Dangerous Liaisons. PNAS. 97: 12953-12955.

Rice, W & B Holland. 1999. Reply to comments on the chase-away model of sexual selection. Evolution. 53: 302-306.

Simmons, LW. 1990. Nuptial feeding in tettigoniids male costs and the rates of fecundity increase. Behavioral Ecology and Sociobiology. 27: 43-47.

Simmons, LW & GA Parker. 1989. Nuptial feeding in insects: Mating effort versus paternal investment. Ethology. 81: 332-343.

Thornhill, R. 1980. Mate choice in *Hylobittacus apicalis* (Insecta: Mecoptera) and its relation to some models of female choice. Evolution. 34: 519-538.

Thornhill, R & J Alcock. 1983. The evolution of insect mating systems. Harvard University Press, Cambridge, MA.

Trivers, R. 1972. Paternal investment and sexual selection. In: sexual selection and the descent of man. Aldine, Chicago, IL.

Tryjanowski, P & M Hromada. 2005. Do males of the great grey shrike, *Lanius excubitor*, trade food for extrapair copulations? Animal Behaviour. 69: 529–533.

Vahed, K. 2007. All that glisters is not gold: sensory bias, sexual conflict and nuptial feeding in insects and spiders. Ethology. 113: 105-127.

Vahed, K. 1998. The function of nuptial feeding in insects: a review of empirical studies. Biol. Rev. 73: 43-78.

Van Schaik, C & CH Janson. 2000. Infanticide by males and its implications. Cambridge University Press, Cambridge, MA.

Voigt CC, R Michener, TH Kunz. 2005. The energetics of trading nuptial gifts for copulations in katydids. Physiological and Biochemical Zoology. 78: 417-423.

Wedell, N. 1993. Mating effort or paternal investment? Incorporation rate and cost of male donations in the wartbiter. Behavioral Ecology and Sociobiology. 32: 239-246.

West-Eberhard, MJ. 2003. Developmental Plasticity and Evolution. Oxford University Press: New York.

Wolfner, MF. 2002. The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. Heredity. 88: 85-93.