

A REVIEW OF SEXUAL CONFLICT THEORY: THE BATTLE OF THE SEXES

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## **Abstract**

Sexual conflict is a process that occurs when the evolutionary interests of the sexes is not aligned. The theory began with Darwin in the 1800's. His observations of sexual dimorphism and traits which did not seem to follow the rules of natural selection led him to the theory of sexual selection. Sexual selection resolved some of the issues which were noted in the evolutionary processes he observed but not all of them. For example, it did not explain traits which increased the male optimum fitness while decreasing the female optimum fitness. It was decades before the concept of sexual conflict was formulated, and even longer before the underlying mechanisms were understood. Sexual conflict is different than sexual selection and there are ways to differentiate which of these processes have occurred in a population.

The main forms of sexual conflict are intralocus and interlocus conflict. These involve the interactions between alleles in the genome of the sexes. Intralocus conflict involves conflict at a single locus in the genome while interlocus conflict involves conflict between different loci. Interlocus conflict has been more extensively studied due to its association with sexually antagonistic coevolution (SAC). SAC draws the attention of scientists due to the possibility it is related to the fundamental biological process of speciation.

Sexual conflict targets certain traits at defined periods in the mating process. These periods include first, the precopulatory stage which is before the act of copulation begins. Second, there is the stage during copulation but before fertilization of the embryo. Finally, there is the postcopulatory postzygotic stage which is after copulation has ended and fertilization has occurred. Each of these points in the process of mating has traits or behaviors which sexual conflict may target.

This review concludes with a proposed experiment to determine if sexual conflict is occurring in a group of four genera of mosquitoes. The experiment utilizes the attributes of sexual conflict to differentiate between other processes. A major component is the consequence of mating systems on selective processes to determine if sexual conflict is involved in the evolution of male accessory gland proteins.

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## Introduction

Sexual conflict is a conflict between the evolutionary interests of the two sexes (Parker 1979). When there are divergent interests in reproduction between males and females, traits favored by one sex may be costly to the other (Chapman et al., 2003). These traits may then be termed sexually antagonistic. The generation of sexually antagonistic adaptations in both sexes may cause a coevolutionary arms race between the sexes (Chapman et al., 2003). This occurs as it is possible that selection may generate a retaliatory change in the sex experiencing less control (Parker 2006). The cyclical and escalating coevolution of reproductive traits in both sexes in the process are experiencing what is termed sexually antagonistic coevolution (SAC) (Arnqvist 2003). SAC is not equivalent to sexual conflict, though it may be a product of it (Parker 2006).

Sexual conflict acts throughout the mating process (Parker 2006). This includes before mating occurs or the precopulatory period (Trivers 1972; Dawkins 1976; Parker 1979). It also occurs during mating when for example male genitalia can injure the female. The next time frame is after mating but before fertilization the postcopulatory/prezygotic period (Thornhill & Alcock 1983). Finally, sexual conflict can occur after the exchange of genetic material has been made and fertilization has occurred the postcopulatory/postzygotic time period (Trivers 1972; Maynard Smith 1977).

Sexual conflict has grown as a concept and been built upon from its early notions first conceived by Darwin in the 1800s (Parker 2006). There was a resurgence of interests in sexual conflict in the 1970's mostly attributed to Trivers (1972) and Dawkins (1976) as they investigated parental investment and selfish genes (Tregenza et al., 2006). In this time area the theoretical basis and interest in clearly defining the concept were sought by one of the areas leading scientists G.A. Parker (Tregenza et al., 2006). Multiple decades then passed before the concept was strongly revisited, and began to reappear in scientific literature (Tregenza et al., 2006). In the late 1990's and in to the current decade technological advances have made it possible to begin to empirically test the concept and provide support for a clearer definition of the term, though there continues to be discrepancy and misuse of the term (Tregenza et al., 2006).

Sexual conflict is often confused with sexual selection. Sexual selection was first noticed as Darwin made observations of traits which should not be selected for by natural selection, yet

persisted in populations (Chapman 2009). In order to make a distinction between sexual selection and sexual conflict, models of coevolution in sexual selection and sexual conflict were generated (Tregenza et al., 2006). One model with regard to sexual selection was the Fisherian Runaway Hypothesis. This hypothesis involves a genetic mechanism for coevolution involving female mating preferences and secondary characteristics of males (Lande 1981). The good genes model is an additional example of a gene based model of sexual selection (Arnqvist et al., 2005). This hypothesis involves female preference for secondary traits as indicators of genetic quality (Arnqvist et al., 2005). This is not a full representation of models which make predictions for the outcome of sexual selection (Paul 2001). As far as sexual conflict, there are also models that predict the outcomes of male and female interaction in sexual conflict, and some of these will be explained in this review. The Gavrilets model (2001) proposes that exaggerated male traits to overcome female traits in mating can result from direct selection on an increase of female resistance to mating. This model contrasts in some aspects with the predictions of typical models of sexual selection (Arnqvist et al., 2005). In all, there are differences and similarities in the genetic outcomes of sexual selection and sexual conflict, and some of them will be explored in this review.

The genetics of sexual conflict and sexual selection should be thought of in terms of gene interaction. Genes can interact either on intralocus or interlocus variation (Parker 2006). Intralocus conflict occurs when selection on a shared trait displaces one sex from its phenotypic optimum (Bonduriansky et al., 2008). Interlocus conflict occurs between sexually antagonistic alleles at different loci. It causes one or both sexes to be displaced from its optimum for a phenotypic trait as a result of selection on the other sex (Bonduriansky et al., 2008). Utilizing an approach focused on gene interaction should allow for a scientist to differentiate between sexual conflict and sexual selection due to the fact that there are different predictions for one versus the other.

The most interesting aspect of the concept of sexual conflict and main reason many biologists are excited about the theory is the possibility that it is an engine of speciation. This hypothesis is supported evidence showing sexual conflict to have the potential to promote allopatric divergence (Rice et al., 1998; Arnqvist et al., 2000). A model designed by Gavrilets (2000) supports the notion that sexual conflict promotes allopatric divergence as it can cause rapid divergence when selection is acting in different directions in different populations (Parker

2006). There are other supported models showing how sexual conflict may be driving speciation including Buridan's Ass where males are trapped in the middle of two diversifying female groups (Parker 2006). Also worth mentioning is the engine of speciation model, in which rapid evolutionary change can occur faster in larger denser populations (Martin & Hosken 2003). Empirical evidence has begun to emerge recently through studies in dipterans showing high dN/dS ratios in reproductive proteins (Swanson et al., 2001; Dottorini et al., 2007). This review explores the history and genetics of sexual conflict, the differences between sexual conflict and sexual selection, and the consequences which may occur due to sexual conflict. It concludes with a proposed experiment to provide additional empirical evidence for higher rates of molecular evolution in genes affected by sexual conflict.

## **CHAPTER 1 - History and Genetics of Sexual Conflict**

The term 'sexual conflict' was first used by G.C. Williams in 1966 (Tregenza et al., 2006). However, the first notion of sexual conflict can be inferred from work as early as 1871 through Charles Darwin (Parker 2006). The theory has gained much support and more is now known about the cause and effects of the force of sexual conflict. Models to support the theory began to be formed in the 1930's with Fisher (1930), Wright (1931), and Haldane (1932) (Chapman 2009). There was then a period during which little progress is noted. Interest was revived in the 70's with Trivers (1972), Dawkins (1976), Parker (1979) and Charnov (1979), though these studies are not entirely inclusive of the advances in this era (Tregenza et al., 2006; Parker 2006). Resurgence occurred again in the 1990s with Rice (1992 and 1996) (Tregenza et al., 2006). This resurgence was likely due to the availability of technology that was being generated during this time period and the ingenuity to apply it to the concept of sexual conflict (Tregenza et al., 2006). In the current decade an immense amount of research has gone into clarifying the definition of sexual conflict, and determining the biological components involved. While there is extensive literature, some contributing studies involved in clarifying the topic include, Arnqvist (2005), Chapman (2003), Parker (2006), and Tregenza (2006). This brief review of contributions is meant to leave the reader with an idea of how the field of sexual conflict theory has advanced and where it currently stands.

### **From Darwin to Modern Assumptions**

The idea of sexual conflict began with the keen observations of reproductive traits by Charles Darwin (Parker 2006). Sexual reproduction was initially thought to be a harmonious interaction benefiting both male and female (Tregenza et al., 2006), Darwin's discoveries of disparity between the preferred outcome of mating interactions in males and females led to new ideas and concepts. In the process of developing his theory of sexual selection he discovered selection on traits which appeared deleterious to survival (Chapman 2009). While his ideas had strong merit much time passed before literature began to reappear addressing this concern.

It was the early 1900's when a gene centered view was taken on sexual selection (Chapman 2009). During this time Fisher, (1915 and 1930), Wright (1931), and Haldane (1932)

created models of sexual selection which provided clarity for the field. By clarifying the theory of sexual selection and causes of evolution these scientists instigated behavioral study at a gene level (Chapman 2009). These advances in sexual selection theory were necessary to unveil the more elusive sexual conflict.

Although the seminal works provided a foundation for sexual conflict research, little less was done over the next three decades. In 1966 Sexual conflict was used as a term for the first time, though the author contributed the idea to Fisher (Williams 1966). It was not until the 1970's when Trivers, through his introduction of parental investment, reignited interest in the field and the term became more widely used (Chapman 2009). He was not alone in making contributions to the field at this time. Dawkins introduced the selfish gene, as well as male female conflict, and coevolution of armaments became a central prediction of sexual conflict theory (Dawkins 1976; Tregenza et al., 2006; Chapman et al., 2003). Parker utilized Trivers (1972) thesis and new information to build on the theory of sexual conflict. He incorporated Trivers ideas with the observations of male-female conflict in reproductive related traits. He argued there would be conflict between the sexes over reproductive decisions (Arnqvist et al., 2005). Studies based upon traits involved in reproductive decisions helped identify evolutionary outcomes for male-female conflict over parental care and male ornamentation (Parker 1979; Tregenza et al., 2006; Parker 2006). Traits were also noticed which were involved in male versus female function (Charnov 1979). The realization here was then that male and female preferences over traits could differ and coevolutionary interests of the sexes were not often aligned (Chapman 2009).

In 1990's there were many models which stemmed from Parkers initial models. These include Gavrilets genetic models, phenotype dependent and phenotype independent models, and non-equilibrium models (Arnqvist et al., 2005). While these models both raised and answered questions about sexual conflict they provided information to begin basing more productive empirical studies. With these models definitions of sexual conflict became narrower. The most popular and widely accepted definitions now include 1) "When the optimal value of a trait differs between the two sexes", 2) "When one sex evolves a trait that does not maximize the fitness of the other sex", 3) "The load created by disruptive selection between the sexes on fitness related character" (Tregenza et al., 2006). There are discrepancies among each of the definitions, though they are generally accepted by the scientific community. These clearer

definitions along with technological advances created opportunities to further advance the field. Rice (1992 and 1996) provided experimental evidence for sexual conflict's evolutionary potential. This occurred through Rice's investigation of sexually antagonistic genes and sexually antagonistic male adaptations (Tregenza et al., 2006). This research revealed it was possible to upset the balance of interests between mates. This allowed sexual conflict to be revealed empirically while illuminating its potentials in regard to evolution (Tregenza et al., 2006)

Current research has made extensive advances in the field of sexual conflict. Through investigation in rates of molecular evolution and speciation, additional evidence strongly supports sexual conflict as a promoter of divergence and an engine of speciation (Arnqvist et al., 2000; Swanson et al., 2001; Dottorini et al., 2007). The success of these studies indicate the necessity to have a clear functional understanding of the traits involved in order to isolate sexual conflict as the force leading to a pattern of rapid evolution of reproductive traits or speciation (Chapman et al., 2003). It has also become apparent there is a need to involve both male and female traits in order to obtain unambiguous results. There are notable studies utilizing traits involved in both sexes, in which the function of sexual conflict is understood (Chapman et al., 2003; Arnqvist et al., 2002; Bergsten et al., 2001). Such studies allow more precise evolutionary inferences to be drawn about trait divergence (Chapman et al., 2003).

### **Sexual Conflict versus Sexual Selection**

A main challenge throughout the history of sexual conflict has been a differentiation between sexual conflict and sexual selection. In order to understand the difference between the processes, let's start by explaining what is sexual selection. Sexual selection was first defined by Darwin as "the advantage which certain individuals have over others of the same sex and species solely in respect to reproduction" (Darwin 1871). Note that sexual conflict fits under this definition. Sexual selection arises from anisogamy, the union of two dissimilar gametes (Parker 2006). Darwin found extravagant male traits hard to understand and fit into the theory of natural selection (Paul 2001). Sexual selection theory explained issues such as sexual dimorphism which could not be fully explained by natural selection (Arnqvist et al., 2005). There are those who consider sexual selection to be a component of natural selection (Endler 1986, Andersson 1994). However, regardless of this argument, sexual selection is well enough defined in order to compare it to sexual conflict.

Darwin considered there to be two mechanisms of sexual selection. One is mate competition and the other mate choice (Paul 2001). Mate competition involves a contest between two members of the same sex in order to remove rivals from the mating scenario; in some cases, this may result in the death of the competing individual (Paul 2001). Mate competition was redefined later as ‘behaviors which expand an individuals potential for mates’ (Wiley and Poston 1996). Mate choice, on the other hand, is between individuals of the opposite sex and involves selection of high quality mates (Paul 2001). This definition has also been revised since Darwin’s initial explanation as ‘any behavior that restricts membership in its potential set of mates’ (Wiley and Poston 1996). Mate choice involves the behaviors of an individual which will restrict members of the opposite sex from being selected as a mate (Paul 2001).

There have been advances in research in the theory of sexual selection which have more clearly defined the mechanisms of sexual selection. They have considered male-male competition and mate choice the main mechanisms for sexual selection with there being several forms of both; included in these mechanisms pertaining to mate competition are endurance rivalry and reproductive suppression. Those categorized as mate choice include precopulatory and postcopulatory choice. For a more complete review of mechanisms for sexual selection see Table I published in Paul 2001 (Parker, 1970; Eberhard, 1996). While male-male competition was widely accepted, the scientific community had difficulty accepting mate choice which indicates females can be moderators of reproduction (Paul 2001). Theories of mate choice are numerous and include direct benefits, the Fisherian runaway hypothesis, and handicap models. A more complete review of these theories can be found in Table II of Paul 2001. In the 1970’s the views of Darwin were accepted and research in this area led to a greater understanding of male and female roles in the evolution of phenotypes. It has also become clear that neither of the two main categories of mechanisms for sexual selection (mate competition and mate choice) are restricted to one sex (Paul 2001). The theory was also able to be used to make predictions about parental investment, operational sex ratio, mutual mate choice, and increased fitness in multiple mating females (Paul 2001).

Sexual conflict is a process which occurs when there are differences in the evolutionary interests of the sexes (Chapman et al., 2003). The process can be separated into intralocus and interlocus conflict (Chapman et al., 2003). These forms of conflict result in a displacement of one or both sexes from their optimum reproduction (Bonduriansky et al., 2008). These forms of

conflict differ from one another based upon where the conflict occurs in the genome of the species. If alleles at a single locus are sexually antagonistic then it is considered intralocus conflict. Conflict between different loci of the sexes will result in interlocus conflict (Bonduriansky et al., 2008). These forms of sexual conflict have the potential to generate sexually antagonistic coevolution (Lessells 2006). This coevolution is capable of leading to the evolution of reproductive novelties, and eventually speciation (Chapman 2009). For example, a male trait such as a seminal fluid protein which has the ability to cause a female to lay eggs and also decrease her lifespan may decrease that female's fitness relative to other females. This may in fact affect the fitness of an entire population as male and female fitness cannot evolve independently (Arnqvist 2003; Fisher 1930). Females may evolve a response to such a male seminal fluid toxin to decrease the negative effects. Such a female response would place selection pressure back on males. This type of sexually antagonistic coevolution not only can lead to novel traits, but result in the formation of new species as the trajectory of sexually antagonistic evolution is expected to follow different paths in different populations.

Given these descriptions of sexual selection and sexual conflict, we are still left with a question, "How do we distinguish between the two processes?" Initially, it appears that sexual selection would always result in a maximization of female fitness relative to other females in the population. For example, sexual selection allows an increase in relative female fitness through mate competition. One of the forms of mate competition, contest competition, allows females to choose the fittest male by discriminating against behavioral displays or physical combats (Paul 2001). On the other hand, sexual conflict may yield deleterious effects on relative female fitness. However, sexual conflict is capable of yielding a scenario where male interests result in maximizing the relative fitness of females (e.g. parental investment) (Lessells 2005). Thus, we are still left with the question of how to differentiate between these two processes.

One way to illuminate differences between these two processes is to assess the affects of mating system on the rates of molecular evolution of reproductive genes. Important to consider here are monogamy and promiscuity. In monogamy, a pair is formed between a male and a female, and they mate only with one another (Ehrlich et al., 1988). Promiscuity is a mating system in which males and females copulate with multiple individuals (Andersson 1994). In order to recognize differences in the occurrence and behavior of the two processes it can be beneficial to utilize separate mating systems as treatment groups (Arnqvist et al., 2000). Sexual

selection should occur in both mating systems while sexual conflict should only occur in promiscuous systems. Sexual selection will act in any system on an allele which may lead to greater relative fitness of females. Sexual conflict will only occur when there is a difference between the fitness optimum of males and females. Molecular evolution describes evolution at the level of DNA. The speed at which changes occur in a genome is what will be referred to here as the rate of molecular evolution (Graur et al., 2000). An indicator of selection on a gene is the dN/dS ratio. This is the number of nonsynonymous substitutions of amino acids (dN) compared to the number of synonymous substitutions of amino acids (dS). When dN exceeds dS, i.e. a dN/dS ratio  $>1$ , this indicates the occurrence of adaptive evolution (Swanson et al., 2001).

To link types of selection and mating systems with patterns of evolution, let's begin by considering the case of a gene not under sexual conflict or sexual selection. In this example there is no reason to expect that mating system alone will affect rates of molecular evolution (Figure 1A). In the case of sexual selection, genes underlying the targeted phenotypes can experience elevated rates of molecular evolution. However, there is no reason to expect that mating system will affect these rates for genes underlying female preference traits. There is an expected effect of high rates of molecular evolution in genes involved in male-male competition as competition is more intense as the sex ratio becomes more male biased (Figure 1, B). Alternatively, in sexual conflict there will be a rapid increase in the rate of molecular evolution as the number of copulations with different partner's increases. This is due to intralocus and interlocus conflict leading to SAC (Chapman et al., 2003). This rapid increase in rates of molecular evolution has been shown empirically by Arnqvist et al., 2000 (Figure 1, C).

In total, what do these expectations say about the difference between sexual selection and sexual conflict? First, we can distinguish female preference genes (i.e. sexual selection genes) from female resistance genes (i.e. sexual conflict genes) (Figure 1B vs. Figure 1C) and thus sexual selection from sexual conflict. What about distinguishing male competition genes (i.e., sexual selection genes) from male persistence genes (i.e. sexual conflict genes) based on rates of molecular evolution? We can do this by looking at fitness effects on females across populations. In a system involving sexual selection, females should achieve maximum fitness in all populations. If the female's fitness was variable, perhaps with some populations showing decreased fitness, this would indicate sexual conflict.

## **Sexual Conflict and Selection on Intra and Interlocus Allelic Variation**

Interactions between nuclear alleles can be intralocus or interlocus (Parker 2006). There are interesting responses at these loci when involved with or affected by sexual conflict or sexual selection. The evolutionary dilemma here is that there are consequences of the sexes sharing the same genome (Bonduriansky et al., 2008). Intralocus sexual conflict is defined as an interaction between alleles at a locus which are sexually antagonistic, and results in one or both sexes being removed from their optimum for a phenotypic trait that affects fitness (Bonduriansky et al., 2008).

Intralocus conflict has not been given the same amount of attention as interlocus conflict due to the difficulty in studying it empirically (Bonduriansky et al., 2008). However, it is not to be ignored as there have been studies indicating its importance as a source for antagonistic variation (Bonduriansky et al., 2008; Chippindale et al., 2001; Chapman et al., 2003).

In relation to sexual conflict, one important trait under intralocus conflict is parental investment. This complex form of conflict has received a great deal of attention and is worthy of further study (Trivers 1974; Macnair and Parker 1978). Parental investment involves a conflict between the male and female for what the offspring should take from the mother (Parker 2006). This conflict is created as the more investment one parent gives the more the other can potentially increase its reproductive success (Trivers 1972). Genomic imprinting is a form of parental investment. It allows expression of alleles to differ depending on whether it is inherited from the male or female, and depending on which parent the allele comes from it may be silenced (Bonduriansky et al., 2008).

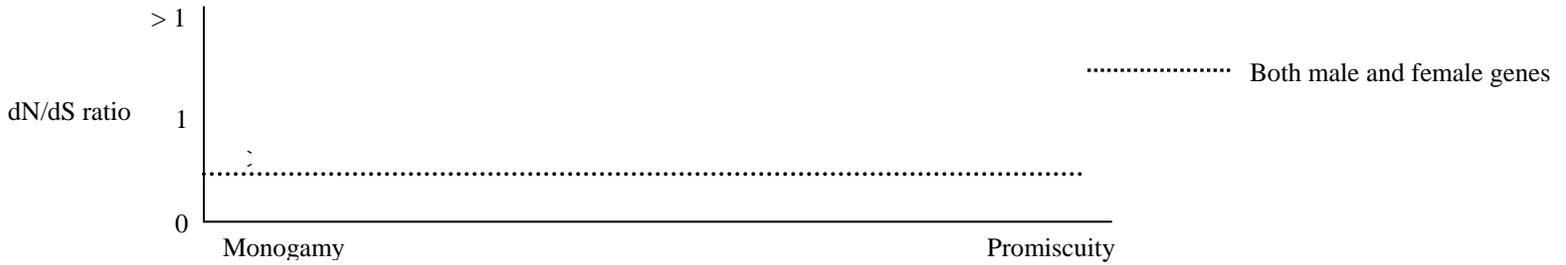
Interlocus conflict is defined similarly to intralocus conflict, with the difference being that the interaction is between alleles at different loci within the genome (Bonduriansky et al., 2008). Intralocus and interlocus conflict are not completely exclusive as interlocus conflict can be mediated by alleles which are at the same locus (intralocus) in males and females (Tregenza et al., 2006). Interlocus conflict has received much more attention than intralocus conflict to date. It has caught the attention of many scientists due to evidence which supports hypotheses that it may be the source of sexually antagonistic coevolution between male and female traits (Chapman et al., 2003). This is accompanied by empirical evidence from Parker (1998) which conveyed that SAC can lead to rapid and unpredictable evolutionary change (Chapman et al., 2003.) There are many biological interactions between phenotypes which fall in this category

(Parker 2006). Characteristics involved in this form of sexual conflict include mating frequency, female reproductive rate, and clutch size (Chapman et al., 2003).

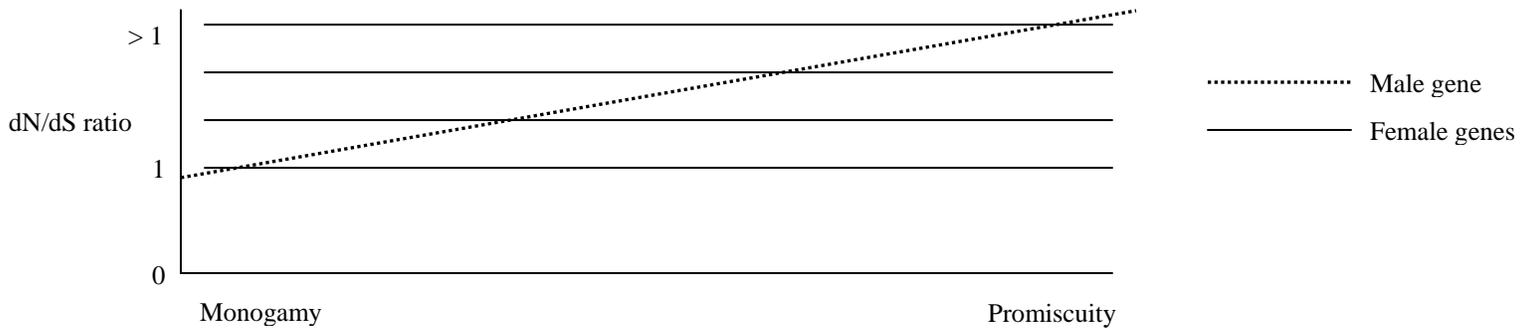
## Figures for Chapter 1

**Figure 1. Predictions about the molecular evolution of individual genes under sexual selection or sexual conflict.**

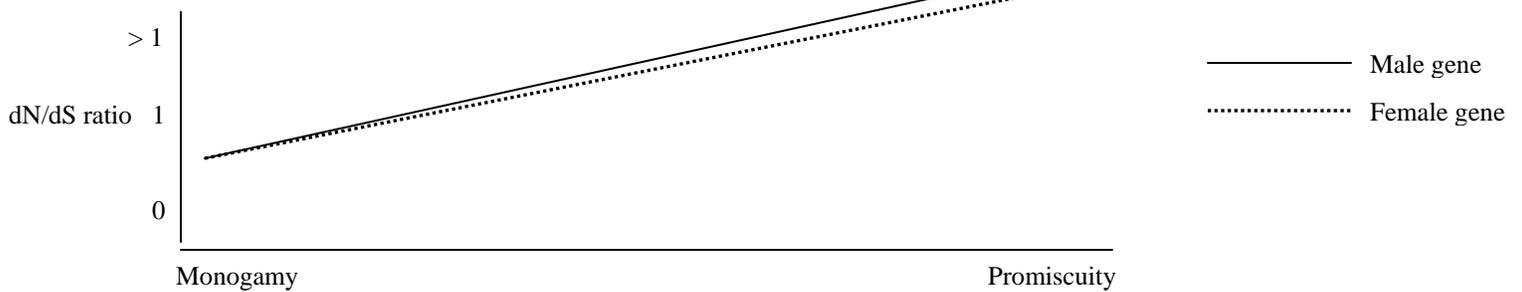
### A) Genes Not Under Sexual Selection or Sexual Conflict



### B) Genes Influenced by Sexual Selection



### C) Genes Influences by Sexual Conflict



**This is a representation of gene evolution under different mating systems and kinds of selection. A) This line represents a male or female gene not under sexual selection or sexual conflict. B) The solid lines represent possible female preference genes under sexual selection. The dashed line represents male-male competition genes under sexual selection. C) The solid line represents a male gene under sexual conflict. The dashed line represents a female gene under sexual conflict.**

## **CHAPTER 2 - Targets of Sexual Conflict**

Sexual conflict is an evolutionary process which targets many different phenotypes. These phenotypes include behaviors which can cause fitness consequences like manipulative and harmful behaviors (Lessells 2006). There are costs and benefits to each of these behaviors which may have evolutionary consequences. Fitness can be positively or negatively affected for one sex or the other depending on the outcome of the behavior. Such behaviors include, but are not limited to, mating conflict, parental investment, limiting resources, infanticide, and mate cannibalism (Parker 2006).

There are traits which sexual conflict targets allowing selection to make evolutionary changes. These include mating decision, ejaculate manipulation, and sperm selection (Parker 2006). These traits can be manipulated at different times in the mating process by both sexes. When considering the time points in the mating process in which conflict occurs there are three prominent areas which will be discussed. First is the precopulatory period which is the time frame before copulation occurs. The second time frame begins with the act of copulation, but ends before the fertilization occurs. The final time frame is the postzygotic period, which is the time after a zygote is formed. These time frames of mating and the behaviors and consequences involved are examined in detail in this chapter.

### **Precopulatory**

Sexual Conflict can occur during a point in the mating process referred to as precopulatory. This is a time period before the act of copulation and transfer of DNA occurs. It has been described as a behavioral pattern that restricts the probability of fertile matings with particular partners (Paul 2001). Precopulatory sexual conflict involves an individual's decision of whether or not to mate and whether a potential mate is suitable. When one sex prefers to mate while the other does not this is a form of precopulatory mating conflict (Parker 2006).

The decision to mate can be based on many factors. A female who has previously mated may suffer additional harm which could potentially decrease her fitness relative to other females (Jennions et al., 1999). The economy of mating involves male fitness being limited by the number of female gametes. This encourages the male to obtain multiple matings in order to achieve a higher fitness (Arnqvist et al., 2005). However, the costs of multiple matings vary

between the sexes, which is why there is conflict over the number of matings which each sex prefers. Mating can have direct costs to both the female and male (Arnqvist et al., 2005). Males can physically harm females, reduce lifespan, or damage the efficiency of the female immune system. Females on the other hand may be cannibalistic to their partners, such as the praying mantis (Arnqvist et al., 2005).

It has been shown that the risk of multiple mating is higher to females and causes resistance to male attempts to mate (Arnqvist et al., 2005). This causes male persistence to evolve and the formation of a coevolutionary arms race (Arnqvist et al., 2005). In order to avoid female resistance males have evolved behaviors to force matings upon females. For example, male bats have been observed to briefly break hibernation in order to inseminate torpid females (Tideman 1982), and in funnel-web spiders where the male anesthetize the female and mate with her unconscious body (Singer et al., 2000).

### **Copulation to Postcopulatory, Prezygotic**

Sexual conflict occurs during the act of copulation itself. Female's fitness may be affected by physical harm by penile spines and conflict over mate duration (Arnqvist et al., 2005). In seed beetles a coevolutionary arms race has occurred between the sexes where males harmful genitalia place pressure on females to increase the amount of connective tissue in their reproductive tract (Ronn et al., 2007). There are also grasping traits in males which stop females from ending the copulation process (Darwin 1871). An example of this occurs in water striders where the males have abdominal claspers which grasp the females during the mating struggle (Arnqvist 1992). The event of copulation in bedbugs involves traumatic insemination. This can involve physical trauma and disease in the female (Siva-Jothy 2006).

Nuptial feeding is involved in the copulatory time frame of mating as well. It is beneficial to females as they have more resources to input in offspring production. It is also possible that nuptial feeding will have a negative effect on females exploiting them to overinvest in parental care. This occurs in many bird species (Arnqvist et al., 2005). Nuptial feeding may encourage females to mate more often; this would benefit males which may have otherwise been rejected.

Postcopulatory (prezygotic) is defined as a time when mechanisms that enable a female to select among the sperm of different males in her reproductive tract can be used (Paul 2001). This is when fertilization conflict occurs. The proteins from the male accessory glands are passed

through seminal fluids and have been studied extensively (Swanson et al., 2001, Dottorini et al., 2007; Panhuis et al., 2006). These proteins can reduce female receptivity to additional mates as well as inducing oviposition (Parker 2006). Postcopulatory conflict can also occur through postmate guarding and mating plugs. There are extensive examples of these behavioral traits in Table 4.1 Arnqvist et al., 2005.

Postcopulatory sexual conflict also involves sperm competition (Arnqvist et al., 2005). Sperm competition is a coevolutionary process. Males have been observed to evolve faster, more aggressive, and longer sperm which are capable of displacing previous males' sperm (Arnqvist et al., 2005). These traits have been noted in insects, birds, and fish (Arnqvist et al., 2005). Females also have an important role in sperm competition which is often referred to as cryptic female choice. The theory of female cryptic choice involves a females' capability to choose between sperm (Arnqvist et al., 2005). There is more than one potential way for the process of female cryptic choice to occur. These include ejaculate manipulation, sperm selection, and differential reproductive investment (Parker 2006). Ejaculate manipulation allows females to physically block sperm from her reproductive tract or to eject it (Pizzari & Birkhead 2000). Sperm selection allows females to select against certain sperm in the reproductive tract or at the egg surface (Zeh & Zeh 1997). Differential reproductive investment allows females to adjust the rate of egg laying or invest more in offspring from favored males (reviewed in Simmons 2001).

There are species such as Gulf pipefish where the male carries the offspring and has control over fertilization of the zygote and whether the embryos are allowed to be carried to term or not. Males of this group are capable of taking amino acids from the brood, and in some cases completely aborting all of a females' eggs. This would be an example of cryptic male choice which can act both at the prezygotic and postzygotic phases (Paczoltz et al., 2010).

### **Postcopulatory, Postzygotic**

The time frame of conflict which occurs after copulation and fertilization will be discussed in this section. This is the point at which postfertilization choice can be made. Postfertilization choice involves allowing certain offspring, from the zygote to the birthed stage, to either be aborted or undergo differential investment (Paul 2001). Some of the behaviors involved are parental care, mate desertion, genomic imprinting, and sex allocation.

It is common for there to be conflict in a system over the amount of parental care and mate desertion (Clutton-Brock 1991). Parental care involves conflict between the sexes as each sex would prefer the other to “work harder” (Chapman et al., 2003). It is often true in a biparental species for males to attempt gaining matings. This conflicts with the female as she is then forced to exert more effort in parental care (Chapman et al., 2003). This conflict has consequences such as female-female aggression. Female-female aggression is an occurrence characterized by a female interfering with male mate attraction by limiting male displays for new mates (Eens et al., 1996). This has been noted in birds such as the house sparrow (Veiga 1990).

Genomic imprinting occurs after fertilization and is the process which allows for example, a female to reduce the expression of an allele from the male in female offspring in order to increase fitness potential for her female offspring (Bonduriansky et al., 2008). Genomic imprinting has also been described as “unequal expression of genes depending on the sex of the parent from which they were inherited” (Arnqvist et al., 2005). If a mutant allele causes elevated rates of maternal provisioning only when it is paternally derived it will be favored by selection. The conflict between male and female arises from this as genes which function as embryo growth promoters will be paternally expressed but maternally silenced. Female resistance may cause genes for embryo growth suppression to be maternally expressed but paternally silenced (Arnqvist et al., 2005).

Sex Allocation is a form of postzygotic conflict which involves altering the sex ratio based on local environment and parent condition (Chapman 2009). When one sex is more costly for the parent to produce than the other sex, selection will favor the parent overproducing the less costly sex (Arnqvist 2009). Sex allocation occurs in many mammals. In order to make the process more clear consider a scenario where it is more costly to a mother to produce sons than daughters. In this scenario a gene which codes for excess female production will be favored. This will then result in a female biased sex ratio (Arnqvist et al., 2005). However, genes coding for excess male production, or perhaps an even sex ratio, will be favored in the male genome if sons have a higher or equal reproductive fitness as compared to daughters. Sexually antagonistic coevolution can then occur in this scenario because females and males have different preferences for offspring sex ratio (Arnqvist et al., 2005).

## **CHAPTER 3 - A Proposed Test of Sexual Conflict in Mosquitoes**

There are different mating strategies utilized by organisms to maximize reproductive fitness. Important here are monandry and polyandry. In a monandrous system females will mate with a single male. In a polyandrous system females will mate with multiple males. There are costs and benefits to each of these systems. These are reviewed in Jennions et al., 1999. Benefits of monandry include decreased risks of parasite or pathogenic infection as well as predation. There will likely be an increase in the males' investment in offspring. Costs of monandry include inadequate amounts of sperm to fertilize all eggs, reduced genetic diversity, and possible genetic incompatibility. These are considered costs as they will likely lower the females' fitness relative to other females in the population. Benefits of polyandry include substantial amounts of sperm, increased genetic diversity, and the opportunity to improve on a previous mate. The costs of polyandry include injury to the female, higher rates of infection by pathogens and parasites, as well as increased rates of predation. Polyandry may also cause evolution in males to revert females to monandry. The above costs and benefits of polyandry can lead to sexual conflict. If the sexes have different optimal mating strategies sexual conflict will occur. Sexual conflict is a direct byproduct of polyandry; it may represent the driving agent of reproductive isolation and subsequent speciation. Coevolution can be caused by females selecting for male traits which will increase their fitness relative to other females in the population. This causes selection pressure on males' to evolve in order to maximize their own fitness as related to other males in the population. The arms race between sexes is hypothesized to increase rates of speciation (Arnqvist et al, 2002). The lack of sexual conflict in monandrous species likely leads to lower rates of divergence in these genera. Evidence provided by Arnqvist et al. indicates speciation rates of families which mate multiply to be four times that of monandrous families. It can also be seen in rodents, sea snails, and primates that rates of molecular evolution increase in polyandrous groups (Ramm et al., 2007; Galindo et al., 2003; Clark et al., 2007). (Figure 1)

### **Hypothesis**

Comparing genes in the serine protease inhibitor superfamily across four genera of mosquito will allow association between the effects if any of monandry versus polyandry on the

rate of molecular evolution of mosquito reproductive proteins. Monandrous genera are expected to have lower rates of molecular evolution in reproductive proteins due to a lack of sexual conflict, while polyandrous genera should have increased rates of molecular evolution in such proteins. It has been shown in *Drosophila melanogaster*, a polyandrous group, that there is increased positive selection on protein coding regions in the male accessory glands (Swanson et al., 2001.) In short I expect genes under the pressure of sperm competition resulting from polyandry will diverge more rapidly than those not under this pressure. An association between mating system and rates of molecular evolution should support this.

### **Mosquito Genera and Species Selection**

Mosquitoes were chosen as the system for this study. They are vectors of disease putting 3.3 billion people at risk of malaria each year (WHO). They are vectors of many other diseases as well such as dengue, yellow fever, and West Nile Virus. Importantly this family displays a mating gradient from monandrous to polyandrous. While generally considered monandrous there are limited degrees of polyandry. Another important factor in choosing this organism is that gene flow and population structure have been studied extensively in this system. The main purpose of choosing this system for a comparative study is to show whether limited degrees of polyandry can elevate rates of molecular evolution within reproductive proteins.

*Anopheles*, *Culex*, *Aedes*, and *Sabethes* have been chosen as the genera on which to conduct my research. These were chosen based on information concerning the observed mating behaviors of each genus in general and species-specific data when available. Monandry appears to be a conserved behavior across *Culicidae*, though there are some genera who exhibit a low frequency of multiple mating.

*Anopheles* is noted to be the most promiscuous of the chosen mosquito genera. Multiple mating in field caught and lab reared anophelins has been reported in multiple studies (Tripet et al., 2003; Helinski et al., 2008; Spielman et al., 1967). Most interesting of these studies is the work done by Marc Klowden in 2001. He was able to show that anopheline mosquitoes do not show refractory behavior associated with MAGs. This comparative study may illuminate why Anophelins do not exhibit the behavior of more monandrous mosquito genera. Klowden points out the importance of this lack of refractory behavior to the drive of transgenes in a population, multiple mating may facilitate spread of the transgenes in a population. From this genera five

species of the 455 existing worldwide have been chosen, *An. Gambiae*, *An. freeborni*, *An. quadrimaculatus*, *An. stephensi*, *An. arabiensis* (Harbach, 2007).

*Culex* was chosen for its behavior of infrequent multiple mating (Craig, 1967). Male accessory glands were implanted in virgins of this species which were then caged with males. There was no sign of insemination in the females. This indicates the MAGs have an effect on this species similar to that of the effect observed in *Ae. aegypti*. There were also genetic marker studies done on this species indicating that only one of two males' sperm was used by the female when confined together and overall multiple insemination was infrequent (Kitzmilller, 1958; Spielman, 1956). Though multiple inseminations are infrequent they do occasionally occur and may initiate sexual conflict. The *Culex* species chosen include *Cu. Tarsalis*, *Cu. Pipiens*, and *Cu. quinquefasciatus*. There are 763 *Culex* species worldwide (Harbach, 2007).

*Aedes aegypti* has been studied quite extensively. There is evidence to suggest a role of MAG secretions in mating as well as indication that these are almost entirely monandrous mosquitoes. This species is noted to have lifetime refractory behavior after a successful mating (Klowden, 2001). It has been shown in studies that *Aedes aegypti* may mate a second time if mating were interrupted or otherwise unsuccessful, but in nature this species infrequently mates multiply (Spielman, 1967). These data suggest that some substance transferred from males during mating induces refractory behavior. Spielman showed through experimentation that there was a definite link between the MAGs and the willingness of the female to remate; as well as seminal loss in multiply mated females. He accomplished this by implanting virgin female bodies with whole tissues from MAGs, testes, or ovaries (Spielman, 1967). Females implanted with MAGs experienced a high percentage of seminal fluid loss from the second male when multiply mated. It was also noted that non virgins had decreased interest in mating and those matings that did occur were short in duration (Spielman, 1967). Thus this candidate follows the gradient of mating systems this study is trying to achieve. *Aedes* species chosen include four of the 363 species, *Ae. Aegypti*, *Ae. albopictus*, *Ae. caspius*, *Ae. Triseriatus* (Harbach, 2007).

*Aedes* and *Culex* generally mate only once but there are exceptions to this rule. For example it has been shown that after females undergo a gonotrophic cycle receptivity can be renewed (Dickinson et al., 1997). It is important to include a species known to be completely monandrous. The genus *Sabethes* is one such candidate. *Sabethes cyaneus* is a mosquito which is interesting in many ways. Both males and females possess iridescent scales and ornaments, while

also doing the only reported courtship ritual in mosquitoes (South et al., 2008). It is curious, however, that if these ornaments are removed the male mosquito appears to have no decrease in fitness while the female does suffer a reduction. The most important aspect of the mosquito behavior in relevance to this study is that females were noted to be completely monandrous throughout life and gonotrophic cycles (South et al., 2008). Based upon this *Sa. cyaneus* and *Sa. chloropterus* have been chosen to represent this genus. There are only 38 species in this genera a possible indication of decreased divergence compared to the other mosquitoes (Harbach, 2007).

These four genera create a gradient in mating behavior from somewhat polyandrous to completely monandrous. Polyandry appears to be basal with monandry developing in the non anopheline groups.

Phylogenetic trees indicate that anopheline mosquitoes are in a different subfamily than the other mosquito genera included in this study. This divergence could explain the differences in *Anopheline* reproductive behavior as compared to the others. With this phylogenic information (Figure 2) I feel confident that there will be interesting evolutionary aspects to observe in relation to the serine protease inhibitors in the male accessory glands (Harbach et al., 2004, 2007) (Shepard et al., 2006) (Krzywinski et al., 2001).

## Gene Selection

The gene family which was chosen for this study was identified in a paper authored by Dottorini et al. (2007). The paper revealed conservation of serine protease inhibitors expressed in the male accessory glands across species. Male accessory glands have many putative functions. They have been identified to have behavioral and physiological effects in *Aedes aegypti*. Examples include changes in flight behavior, responses to host cues, oviposition, fertility, ovarian development, blood digestion, and refractory sexual behavior (Sirot et al., 2008). They may carry antimicrobial agents; they may stimulate the number and development of eggs, and also may include noxious chemicals. These are believed to have evolved due to sexual conflict and generally function to increase male fitness (Gillot, 2003). These are important areas of mosquito biology and physiology which must be understood to battle the mosquito as a vector of disease.

In the MAGs an important gene superfamily has been noted. Serine protease inhibitors have many vital functions. They have been associated with fertility in mammals and likely

function as a protein harmful to the females in *Drosophila melanogaster*. Allelic variation in gene Acp62F in *Drosophila melanogaster* is associated with sperm competition and fecundity of mating partners (Dottorini et al., 2007). This gene was shown to have high dN/dS ratios (Swanson et al., 2001). Monogamous species do not exhibit sperm competition. Therefore it is not likely these genes are under the influence of sexual conflict. Comparisons across mosquito genera of this gene superfamily show gene expansion in mosquitoes. I expect the comparison to show that the serine protease superfamily proteins, in the male accessory glands, chosen for this study are diverging more rapidly in the polyandrous genera in comparison to monandrous genera.

## **Materials and Methods**

Sequence data from sequenced genomes are available for three species *An. gambiae*, *Ae. Aegypti*, and *Cu. quinquefasciatus*. These will be taken from databases such as Vectorbase and NCBI. For the other 11 species preserved male specimens will be utilized for RNA extraction from male accessory glands. These specimens have come from lab reared colonies across the world. Tissues will be dissected directly from the male reproductive tract (MAGs and testis). RNA isolation will be performed using the RNAqueous kit. The RNA will provide a template for 5' RACE with degenerate primers designed from the alignment in the Dottorini et al., 2007 paper (Figure 3). The RLM 5' RACE kit was utilized for this procedure. Gel extraction with Quiagen kits will be performed on bands of the correct size. Sequencing will be direct or from clones and will be sequenced at the on campus sequencing facility. JOVE protocol was used for rearing of the *Culex* mosquitoes (Das et al., 2007).

## **Analysis**

Each of the genes in this superfamily which are isolated from the mosquitoes will be compared across the four genera of mosquitoes *Anopheles*, *Aedes*, *Culex*, and *Sabethes*. My goal will be a total of fourteen species between the four genera, although technical issues may limit the final number. Alignment and Phylogeny will be performed in order to look at rates of synonymous and nonsynonymous amino acid substitutions on branches of the phylogeny between genera as well as for each mosquito clade. The dN/dS ratios will be calculated. The homologs will be mapped on a phylogenetic tree to determine evolutionary relatedness. This will

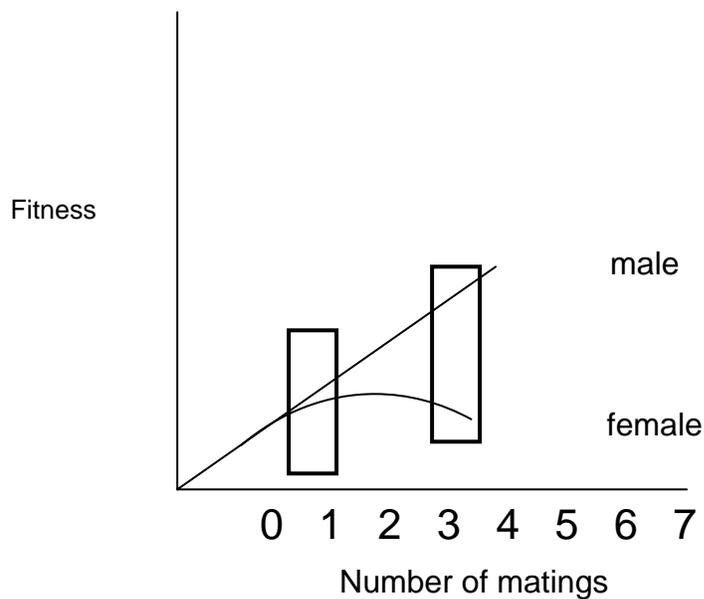
assist in determining divergence of the superfamily across genera. Finally, programs such as PAML and Datamonkey will be used to further assess which, if any, codons are under positive selection.

## **Conclusion**

This comparative study will attempt to show that monandry and polyandry as mating systems have an effect on the evolution of reproductive proteins. I expect the phylogeny to show that increased promiscuity is directly related to rapid divergence of MAG proteins across the family. This study should answer the interesting question; do limited degrees of polyandry manipulate rates of molecular evolution (Figure 2)?

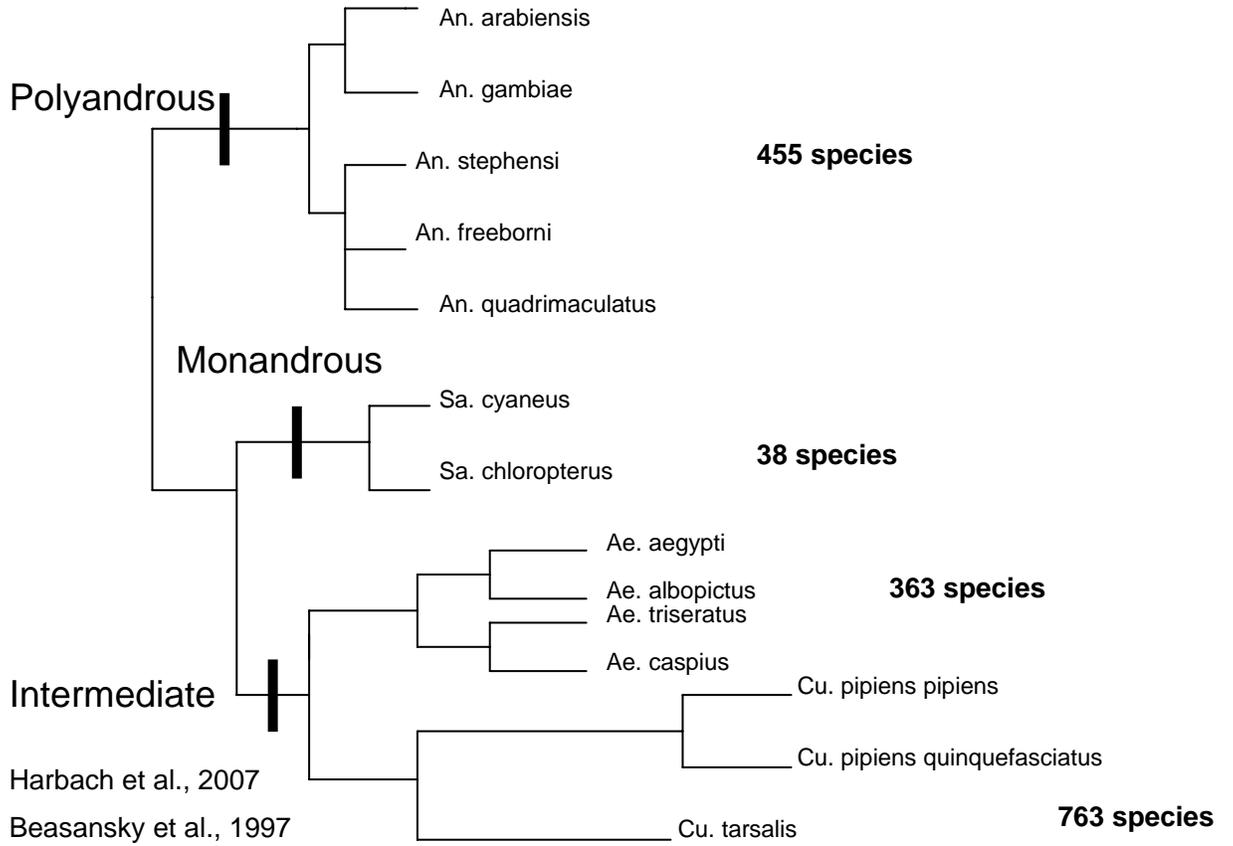
## Figures

**Figure 2. A comparison of fitness between males and females in regard to number of matings.**



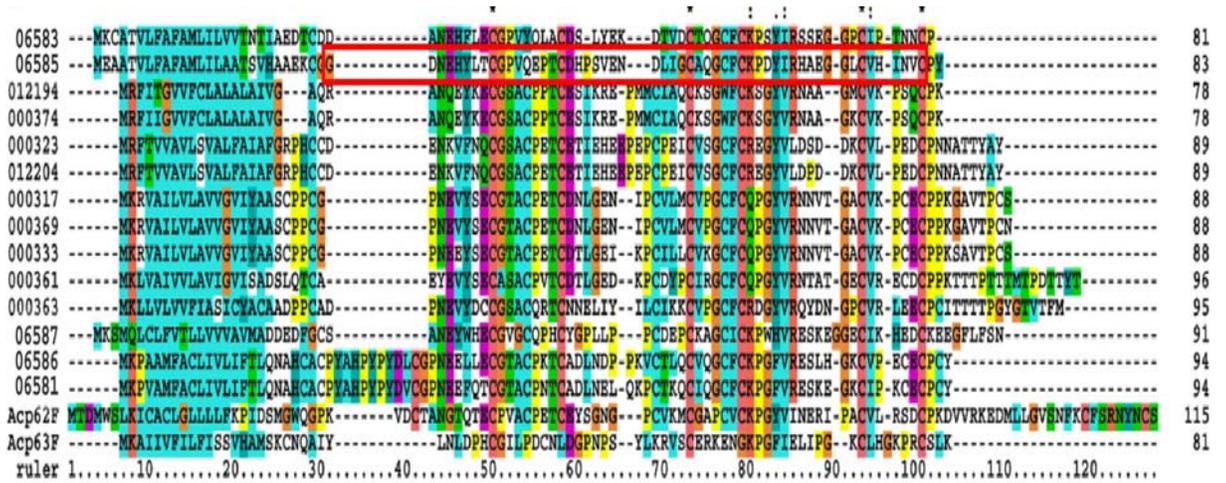
**This figure indicates the limited degree of polyandry being observed in this study as compared to other studied which have been done on a larger scale. It also represents the difference in male and female fitness in relation to the number of matings. Males increase in fitness with additional matings while females have a threshold.**

**Figure 3. A dendrogram of the genera and species to be included in the proposed experiment.**



**This figure depicts a dendrogram of the genera and species includes in this study as well as the total number of species in each genera, and the mating system for each clade.**

**Figure 4. An alignment for experiment primer design.**



This figure shows the alignment of serine protease inhibitors in *An. gambiae*, *Ae. Aegypti*, and *D. melanogaster*. This alignment was the basis for designing the original primers.

## References

- Andersson, M., 1994. *Sexual Selection*. Princeton University Press. New Jersey.
- Arnqvist, G. 1992. Precopulatory fighting in a water strider: Intersexual conflict or mate assessment? *Animal Behavior* 43: 559-567.
- Arnqvist, G., Edvardson, M., Friberg, U., Nilsson, T., 2000. Sexual conflict promotes speciation in insects. *PNAS* 97: 10460-10464.
- Arnqvist, G., Rowe, L., 2002. Antagonistic coevolution between the sexes in a group of insects. *Nature* 415: 787-789.
- Arnqvist, G., Rowe L., 2005. *Sexual Conflict*. Princeton University Press. Princeton and Oxford.
- Arnqvist, G., Nilsson, T., 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Animal behavior* 60: 145-164.
- Arnqvist, G., 2003. Sexual conflict and sexual selection. Lost in the chase. *Evolution*: 58(6): 1383-1388.
- Bergsten, J. et al. (2001) Intraspecific variation and intersexual correlation in secondary sexual characters of three diving beetles (Coleoptera: Dytiscidae). *Biol. J. Linn. Soc.* 73, 221–232
- Besansky, N., Fahey, G., 1997. Utility of the white gene in estimating phylogenetic relationships among mosquitoes. *Mol. Biol. Evol* 14, 442-454.
- Bonduriansky, R., Chenoweth, S., 2008. Intralocus sexual conflict. *Cell Press* doi: 10.1016
- Chapman, T., Arnqvist, G., Bangham, J., Rowe, L., 2003. *Sexual Conflict*. *Trends in Ecology and Evolution*. 18:1
- Chapman, T., 2009. Sexual conflict and Sec allocation. *Biology letters*. 5: 660-662
- Charnov, E. L., 1979. Simultaneous hermaphroditism and sexual selection. *PNAS USA*. 76: 2480- 2484.
- Chippindale, A.K. et al. 2001. Negative genetic correlation for adult fitness between the sexes reveals ontogenetic conflict in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1671–1675
- Clark, Swanson. 2005. Pervasive adaptive evolution in the primate seminal proteins. *PLOS genetics* 1, 35-42.
- Clutton-Brock, T.H. (1991) *The Evolution of Parental Care*, Princeton University Press

- Craig, G., 1967. Mosquitoes: Female monogamy induced by male accessory gland substance. *Science*, 156: 1499-1501.
- Darwin, C., 1871. *The descent of man, and selection in relation to sex*. Murray, London.
- Das, S., Garver, L., Dimopoulos, G. 2007. Protocol for mosquito rearing (*A. gambiae*). Malaria Research institute, Bloomberg School of Public Health, Johns Hopkins University. Cellular Biology issue 5.
- Dawkins, R. 1976. *The selfish gene*. Oxford: Oxford University Press.
- Dickinson, J.M., Klöwden, M.J. Reduced transfer of male accessory gland proteins and monandry in female *Aedes aegypti* mosquitoes. *Journal of vector ecology* 22(1):95-98.
- Dottorini, T., Nicolaides, L., Ranson, H., Rogers, D., Crisanti, A., Catteruccia, F. 2007. A genome wide analysis in *Anopheles gambiae* mosquitoes reveals 46 male accessory gland genes, possible modulators of female behavior. *PNAS* 104: 16215-16220.
- Eberhard, W. G. 1996 *Female control: sexual selection by cryptic female choice*. Princeton, NJ: Princeton University Press.
- Eens, M. and Pinxten, R., 1996. Female European starlings increase their copulation solicitation rate when faced with the risk of polygyny. *Anim. Behav.* 51, 1141±1147
- Ehrlich, P., Dobkin, D., Wheye, D., 1998. Monogamy Polygyny and Natural selection.
- Endler, J. A., 1986. *Natural selection in the wild*. Princeton University Press, Princeton, NJ.
- Fisher, R. A. (1915). The evolution of sexual preference. *Eugen. Rev.* 7: 184–192.
- Fisher, R. A. 1930. *The genetical theory of natural selection*. New York: Dover.
- Galindo, Vaquier, Swanson. 2003. Positive selection in the egg receptor for abalone sperm lysine. *PNAS* 100, 4639-4643.
- Gillot, C., 2002. Male accessory gland secretions: modulators of female Reproductive Physiology and behavior. *Annual Review of entomology* 48: 163-184.
- Graur, D. and Li, W.-H. (2000). *Fundamentals of molecular evolution*. Sinauer
- Haldane, J.B.S. 1932. *The causes of evolution*. London UK: Longmans
- Harbach, R., 2007. The culicidae (Diptera): A review of taxonomy, classification and phylogeny. *Zootaxa* 1668: 591-638.

- Harbach R., 2004. The classification of the genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. *Bulletin of entomological research* 94: 537-553.
- Helinski, M., Hood, R., Knols, B., 2008. A stable isotope dual-labeling approach to detect multiple insemination in un-irradiated and irradiated *Anopheles arabiensis* mosquitoes. *Parasites and Vectors* 1:9
- Jennions, M., Petrie, M., 1999. Why do females mate multiply? A review of the genetic benefits. *Biol Review* 75: 21-64.
- Kitzmilller, J.B., Laven, H., 1958. Tests for multiple fertilization in *Culex* mosquitoes by the use of genetic markers. *Amer. J. Hyg.* 67, 207-213.
- Klowden, M., 2001. Sexual receptivity in *Anopheles gambiae* mosquitoes: absence of control by male accessory gland substances. *Journal of Insect Physiology* 47, 661-666.
- Krzywinski, J., Wilkerson, R., Besansky, N., 2001. Toward understanding Anopheline (Diptera culicidae) Phylogeny: Insights from Nuclear Single copy genes and the weight of evidence.
- Lande, R., 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. USA* 78:3721–3725.
- Lessells, C. M. 2005. Why are males bad for females? Models for the evolution of damaging male mating behavior. *Am.Nat.* 165, S46–S63.
- Lessells, M. 2006. The evolutionary outcome of sexual conflict. *Phil. Trans. R. Soc. B* 361, 301–317.
- Macnair, M. R. & Parker, G. A. 1978 Models of parent–offspring conflict. II. Promiscuity. *Anim. Behav.* 26,111–122.
- Martin, O. Y. & Hosken, D. J. 2003 The evolution of reproductive isolation through sexual conflict. *Nature* 423, 979–982.
- Maynard Smith, J. 1977 Parental investment—a prospective analysis. *Anim. Behav.* 25, 1–9.
- Mitchell, 2002. Higher-level phylogeny of mosquitoes (Diptera: Culicidae): mtDNA data support a derived placement for Toxorhynchites. *Insect systematics and evolution* 33, 163-174.
- Paczoltz, K., Jones, A., 2010. Post copulatory sexual selection and sexual conflict in the evolution of male pregnancy. *Nature* 464: 401-405.
- Panhuis, T., Clark, N., Swanson, W. 2006. Rapid evolution of reproductive proteins in abalone and *Drosophila*. *Phil. Trans. R. Soc. B* 361:261-268;

- Parker, G. A. 1970. The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae) V. The female's behavior at the oviposition site. *Behaviour* 37, 140–168.
- Parker, G. A. 1970b The reproductive behavior and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae) IV. Epigamic recognition and competition between males for the possession of females. *Behaviour* 37, 113–139.
- Parker, G. A., 1979. Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects*. pp. 123–166. London: Academic Press.
- Parker, G. A. & Partridge, L. 1998. Sexual conflict and speciation. *Phil. Trans. R. Soc. B* 353, 261–274.
- Parker, G. A. 2006. Sexual conflict over mating and fertilization: an overview. *Phil. Trans. R. Soc. B* 361,235–259.
- Paul, A., 2001. Sexual selection and Mate choice. *International Journal of Primatology* 23: 877-904.
- Pizzari, T. & Birkhead, T. R. 2002. The sexually-selected sperm hypothesis: sex-biased inheritance and sexual antagonism. *Biol. Rev.* 77, 183–209.
- Ramm, S., Oliver, P., Ponting, C., Stockley, P., Emes, R. Sexual selection and the adaptive evolution of mammalian ejaculate proteins. *Molec Biol and Evol* 25, 207-219.
- Rice, W. R. 1992 Sexually antagonistic genes—experimental evidence. *Science* 256, 1436–1439.
- Rice, W. R. 1996 Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* 381, 232–234.
- Rice, W. R. 1998. Intergenomic conflict, interlocus antagonistic coevolution, and the evolution of reproductive isolation. In *Endless forms* (ed. D. J. Howard & S. H. Berlocher), pp. 261–270. New York: Oxford University Press.
- Ronn, J., Katvala, M., Arnqvist, G., 2007. Coevolution between harmful male genitalia and female resistance in seed beetles. *PNAS* 104: 10921-10925.
- Shepard, J., Andreas, T., Vossbrinck., 2006. Molecular phylogeny and evolutionary relationships among mosquitoes (Diptera: Culicidae) from the northeast United States based on small subunit ribosomal DNA (18S rDNA) sequences. *Journal of medical entomology* 43(3): 443-454.
- Simmons, L. W. 2001 *Sperm competition and its evolutionary consequences in the insects*. Princeton, NJ: Princeton University Press.

- Singer, F., Reichert, S.E., Xu, H.F., Morris, A. W., Becker, E., Hale, J. A., Nouredine, M. A., 2000. Analysis of courtship success in the funnel web spider *Agelenopsis aperta*. *Behavior* 137:93-117
- Sirot, L., Poulson, R., McKenna, M., Girnary, H., Wolfner, M., Harrington, L., 2008. Identity and transfer of male reproductive gland proteins of the dengue vector mosquito, *Aedes aegypti*: Potential tools for control of female feeding and reproduction. *Insect Biochemistry and Molecular Biology* 38, 176-189.
- Siva-Jothy, M. T. 2006. Trauma, disease and collateral damage: conflict in cimicids. *Phil. Trans. R. Soc. B* 361, 269–275.
- South, S., Arnqvist, G., 2008. Evidence of monandry in a mosquito (*Sabethes cyaneus*) with elaborate ornaments in both sexes. *J Insect Behav* 21, 451-459.
- Spielman, A., Leahy, SR. M.G., Skaff, V., 1967. Seminal loss in repeatedly mated female *Aedes aegypti*. *Biol Bull* 132, 404-412.
- Swanson, W., Clark, A., Waldrip-Dall, H., Wolfner, N., Aqudro, C., 2001. Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. *PNAS*. 98, 7375-7379.
- Thornhill, R. & Alcock, J. 1983. The evolution of insect mating systems. Cambridge, MA: Harvard University Press.
- Tidemann, C. R., 1982. Sex differences in seasonal changes of brown adipose tissue and activity of the Australian vespertilionid bat *Eptesicus vulturinus*. *Australian journal of Zoology* 30:15-22
- Tripet, F., Toure, Y., Dolo, G., Lanzaro, G., 2003. Frequency of multiple insemination in field-collected *Anopheles gambiae* females revealed by DNA analysis of transferred sperm. *Am. J. Trop. Med. Hyg.* 68, 1-5.
- Trivers, R. L. 1972. Parental investment and sexual selection. In *Sexual selection and the descent of man*. 1871–1971 pp. 136–172. Chicago: Aldine-Atherton.
- Trivers, R. L. 1974. Parent–offspring conflict. *Am. Zool.* 14,249–264.
- Tregenza, T., Wedell, N., Chapman, T., 2006. Introduction sexual conflict. A new paradigm? *Phil. Trans. R. Soc. B* 2006 **361**, 229-234
- Veiga, J.P., 1990. Sexual conflict in the house sparrow: interference between polygynously mated females versus asymmetric male investment. *Behav. Ecol. Sociobiol.* 27, 345±350
- Wiley, R. H., and Poston, J. (1996). Indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution* 50: 1371–1381.

Williams, G. C. 1966 Adaptation and natural selection. Princeton, NJ: Princeton University Press.

Wright, S., 1931. Evolution in Mendelian Populations. *Genetics* 16:97-159

Zeh, J. A. & Zeh, D. W. 1997. The evolution of polyandry II: post-copulatory defenses against genetic incompatibility. *Proc. R. Soc. B* 264, 69–75. (doi:10.1098/rspb.1997.0010)

[www.entm.purdue.edu/public\\_health/print/insects/mosquito.html](http://www.entm.purdue.edu/public_health/print/insects/mosquito.html)

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