ASSESSING TERRITORIALITY AS A COMPONENT OF MALE SEXUAL FITNESS IN DROSOPHILA SERRATA

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ABSTRACT

While the phenotypic effects of sexual selection have been well studied, the consequences for population mean fitness remain unclear. Additionally, there is a need to more fully characterize how various forms of inter- and intrasexual selection combine to affect the evolution of traits under sexual selection. Here, I address these issues as they relate to male territoriality in *Drosophila serrata*, a model system for the study of female preference for male pheromones. First, I demonstrate that territoriality occurs and is a likely component of male sexual fitness. Results from a phenotypic manipulation indicate that territorial success was also condition-dependent, and that sexual selection against low condition males tended to be stronger given a high opportunity for territory defense. Territorial success depended on body size but not on pheromones. How this and other components of male mating success interact to affect trait evolution and population mean fitness remains an important area for future study.

RÉSUMÉ

Bien que les effets phénotypiques de la sélection sexuelle ont été bien étudiés, les conséquences sur la valeur sélective moyenne de la population restent à élucider. De plus, nous avons besoin de caractériser plus précisemment comment des formes variées de sélection inter- et intra-sexuelles intéragissent pour influencer l'évolution des traits sous sélection sexuelle. J'explore ici ces problèmes en relation avec la territorialité des mâles chez *Drosophila serrata* comme système modèle de l'étude de la préference des femelles pour les pheromones mâles. Premièrement, je démontré que la territorialité est présente et est une composante de la valeur selective sexuelle des mâles. Les résultats provenant d'une manipulation phénotypique indiquent que le succès territorial dépendait de la condition, et que la sélection sexuelle contre les mâles de moindre condition tendait à être plus forte étant donné une grande opportunité de defense de territoire. Le succès territorial dépend de la taille du corps mais pas des phéromones. La façon dont celà ainsi que d'autres composantes du succès reproducteur des males intéragissent pour influencer l'évolution de trait et la valeur sélective moyenne de la population restent un domaine important pour de futures recherches.

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CHAPTER 1

An Overview of Sexual Selection

INTRODUCTION

Sexual selection is a process that arises from variation among individuals in reproductive success, and can occur via two main mechanisms: intrasexual competition, in which individuals (generally males) compete directly or indirectly for access to females, and intersexual mate choice, in which individuals (generally females) mate non-randomly based on preference for certain trait values in their partners (Darwin 1859, 1871). Competition among males has led to the evolution of a variety of traits that improve access to females, including weaponry used in male-male combat and traits involved in sperm competition, whereas female choice typically results in the evolution of exaggerated ornaments and courtship behaviours. Sexual selection has also been found to contribute to other important processes in nature such as diversification within (e.g. sexual dimorphism) and among populations, as well as the origin of new species (Panhuis et al. 2001; Bonduriansky 2011).

Variation in sexual fitness among males may arise from differential success at any of several stages of reproduction (Andersson 1994; Whitlock and Agrawal 2009). The first step necessarily involves searching for and locating a female, and males often compete indirectly when doing so in a process known as scramble competition. Competition at this stage may favour males with more well-developed sensory and locomotor systems, in addition to earlier maturation (Dickinson 1992; Andersson and Iwasa 1996). Direct, contest competition may also occur among males over access to females, or over resources that are needed to attract females. This form of competition has led to the evolution of some of the most conspicuous secondary sex characteristics found in nature including large body size, threat signals, and elaborate weaponry (Clutton-Brock and Albon 1979; Andersson 1994; Emlen and Nijhout 2000).

Finally, once a male has gained access to a female he may have to convince or coerce her into mating and, having done so, may then experience postcopulatory competition with the sperm of other males (Andersson 1994). This has led to the evolution of exaggerated ornaments and displays important to female choice (Ryan and Keddy-Hector 1992; Andersson 1994), as well as such traits and behaviours as nuptial feeding, mate guarding, and selection on sperm size and motility (Andersson 1994; Vahed 1998; Parker and Pizzari 2000).

While sexual selection research has primarily been aimed at understanding the phenotypic effects arising from this process, including those discussed above, several important issues remain less well understood. First, while increased attention has recently been given to understanding the consequences of sexual selection for population mean fitness, a thorough knowledge of how the individual components of male mating success may contribute to this process is lacking. In addition, there remains a need to more fully characterize how various forms of inter- and intrasexual selection act together to determine the total variance in male sexual fitness, and also how these components combine to affect the evolution of traits under sexual selection. In this thesis, I aim to address each of these issues as they relate to territoriality as a component of male sexual fitness.

TERRITORIALITY, SEXUAL SELECTION AND MUTATION LOAD

As gaining access to females is an essential component of male reproductive success, males of many species engage in direct, contest competition with other males to acquire and defend resources that are required by females (Emlen and Oring 1977; Andersson 1994). Such territorial behaviour may be thought of as a form of social dominance, wherein the dominant individual has priority access to resources or any commodity that increases fitness, be it physical (e.g. food) or social (e.g. receptive mates; Kaufmann 1984). Although definitions of what constitutes a territory may differ, for example from a behavioural to ecological context, territoriality can be operationally defined based on observed defense of an area, including behaviours such as displays, fights, chases, and other aggressive interactions between conspecifics (reviewed in Maher and Lott 1995).

In general, males that are able to control access to resources that are required by females will gain fitness benefits via an increased number of matings (Emlen and Oring 1977). This has been well documented in a wide variety of taxa (e.g., insects, Dow and von Schilcher 1975; amphibians, Wells 1977; fish, Warner and Hoffman 1980; birds, Hasselquist 1998; mammals, Carranza et al. 1990). A male's ability to control a territory, and to subsequently benefit from this increased access to females, can be predicted by any of several factors including asymmetries in body size, signals, weaponry, age, and arrival date (Kelly 2008). Importantly, condition, which can be defined as "the relative capacity to maintain optimal functionality of vital systems within the body" (Hill 2011) is also likely to be a strong predictor of territorial success due to the significant investment of time and energy that is generally required to acquire and defend a territory (Grantner and Taborsky 1998; Kelly

2008). Variation in this component of mating success may therefore be expected to favour males in relatively high condition, with condition itself affected by both an individual's genetic quality and the environment they have experienced (e.g., resource availability). This may have important consequences for population mean fitness.

This interest in understanding the population genetic consequences of sexual selection, particularly in relation to its role in reducing mutation load, has seen relatively recent renewal (Whitlock 2000; Agrawal 2001, reviewed in Whitlock and Agrawal 2009). While natural selection is expected to lead to adaptive responses, sexual selection has, by contrast, often been thought of as acting to reduce population mean fitness as a result of the evolution of costly sexual displays and preferences for these displays (Lande 1980), in addition to its role in generating sexual conflict (Pizzari and Snook 2003; Arnqvist and Rowe 2005; Pischedda and Chippindale 2006). However, provided that individuals with reduced non-sexual fitness also have lower reproductive success (i.e. sexual fitness), sexual selection may act to reinforce natural selection, potentially promoting adaptation and increasing population mean fitness (Whitlock 2000; Lorch et al. 2003; Whitlock and Agrawal 2009). Indeed, a reduction in the frequency of particular segregating deleterious alleles as a result of sexual selection has been demonstrated in several recent studies (Radwan 2004; Sharp and Agrawal 2008; Hollis et al. 2009), although others have revealed little effect or have even suggested a cost (Holland and Rice 1999; Hollis and Houle 2011; Arbuthnott and Rundle 2012). What remains less well understood is how different components of sexual selection may contribute to these effects.

Studies that have partitioned total sexual selection to examine certain components have generally reported results in line with those discussed above, with selection acting to improve population mean fitness. For example, search effort (MacLellan et al. 2009) and offensive sperm competition (Clark et al. 2012) have each been found to contribute to selection against deleterious alleles and low condition males, respectively. A thorough understanding of how other important components of male reproductive success might contribute to this process is still, however, lacking, and one goal of this thesis is to examine how variation in territorial success affects selection against low condition males in a laboratory population of the Australian fruit fly *Drosophila serrata.*

In addition to the lekking *Drosophila* species endemic to Hawaii (Spieth 1974; Boake 1989), territoriality has previously been demonstrated in both *D. melanogaster* and *D. simulans* males (Jacobs 1960; Dow and von Schilcher 1975; Hoffmann 1987). Given an environment with discrete food sources, males display highly structured aggressive behaviours including wing threats and vibrations, chasing, quick charges, boxing, and “tussles” with the forelegs. These interactions generally lead to stable dominance relationships, with “winners” occupying the resource at the exclusion of other males and subsequently benefitting from increased mating success (Dow and von Schilcher 1975; Hoffmann 1987; Chen et al. 2002). As territorial behaviour in *Drosophila* is therefore straightforward to observe and quantify in the laboratory, I am able to test whether territoriality occurs among *D. serrata* males, to determine whether success in territory defense is condition-dependent and, if so, whether sexual selection against low condition males is stronger given the inclusion of territory defense as a component of male mating success.

SELECTION ARISING FROM MALE-MALE COMPETITION AND FEMALE CHOICE

Traditionally, male-male competition and female mate choice have been thought of as two distinct processes, the former influencing the evolution of traits that improve access to females, including weaponry used in male-male combat, and the latter driving the evolution of exaggerated ornaments and behavioural displays (Darwin 1871). For studies which have considered these processes together, they have frequently been assumed to work in concert, with female choice facilitated by competition among males (Andersson 1994). This assumption likely relates to the expectation that competitive success is always indicative of a higher quality male, with females thereby able to maximize their fitness by choosing such males for mating (Andersson 1994; Berglund et al. 1996). Traits that reflect dominance, such as large body size and weaponry, are therefore also expected to be signals used in female mate choice (Berglund et al. 1996; Qvarnstrom and Forsgren 1998). More recently, however, a growing number of studies have suggested that male-male competition and female mate choice may in fact be counteracting, with dominant males not always being those preferred by females (Moore and Moore 1999; Casalini et al. 2009).

Particularly for species in which males compete over territories, competition may act either to facilitate female choice by excluding inferior males, or to hamper choice by excluding males that females might otherwise prefer (Wong and Candolin 2005). This latter observation is based on the fact that male dominance is not always predictive of better genes or higher parental quality, and the costs associated with mating with more dominant males may outweigh the potential benefits (Qvarnstrom and Forsgren 1998). Such a situation may be particularly likely to occur when the interests of females and males diverge, leading to an evolutionary conflict between the sexes (i.e. sexual conflict; Moore et al. 2001; Arnqvist and Rowe 2005; Casalini et al. 2009). As the selection imposed by each component of sexual fitness may therefore frequently differ in strength and form, with important consequences for trait evolution as well as fundamental questions such as the maintenance of genetic variation, the relative contribution of each should be quantified to generate a more complete understanding of how sexually selected traits evolve (Qvarnstrom and Forsgren 1998; Hunt et al. 2009).

In *D. serrata*, female mate choice has been well characterized and is known to be based in part on a suite of long chain cuticular hydrocarbons (CHCs) that act as contact pheromones (Chenoweth and Blows 2003, 2005; Howard et al. 2003; Rundle et al. 2009; Delcourt et al. 2010; Sztepanacz and Rundle 2012). In addition, CHCs are known to be important to other aspects of social communication in this (Petfield et al. 2005) and other *Drosophila* species, including functioning to regulate aggression in *D. melanogaster* (Wang et al. 2011). If the outcome of male-male competition in *D. serrata* is also influenced by variation in CHCs, then the opportunity exists to quantify the joint contribution of both processes (i.e. male-male competition and female mate choice) to selection on these traits.

THESIS OVERVIEW

In this thesis, I aim to assess territoriality as a component of male sexual fitness in a laboratory population of the Australian fruit fly, *Drosophila serrata*. Although not yet documented in *D. serrata,* territorial behaviour has been well studied in *D. melanogaster* (Jacobs 1960; Dow and Schilcher 1975; Hoffmann 1987) and can be observed and quantified in the laboratory. I am therefore able to test whether territoriality occurs among *D. serrata* males, to determine whether success in territory defense is condition-dependent, and to assess whether selection against low condition males is stronger given the inclusion of this component of male mating success. This will contribute to our understanding of the consequences of sexual selection for population mean fitness, particularly with respect to how the various components of male mating success may act to reduce mutation load.

Secondly, I address how male-male competition and female mate choice combine to affect the evolution of sexually selected traits in *D. serrata*. Female preference for male CHCs has previously been well characterized (Chenoweth and Blows 2003, 2005; Howard et al. 2003; Rundle et al. 2009; Delcourt et al. 2010; Sztepanacz and Rundle 2012), and I here examined how this component of female mate choice, as well as male-male territorial competition, contribute to selection on male CHCs and body size. This study will help to further our understanding of how inter- and intrasexual selection combine to affect phenotypic evolution.

The following chapter is written in the style of a stand-alone manuscript to be submitted to an academic journal. Despite some resulting overlap in the background material presented, I attempted to keep this to a minimum.

Chapter 2

Assessing the Effects of Territoriality on Male Sexual Fitness in *Drosophila serrata*

INTRODUCTION

Sexual selection is a process that arises from variation among individuals in reproductive success, and can occur via two main mechanisms: intrasexual competition, in which individuals (generally males) compete for access to females, and intersexual mate choice, in which individuals (generally females) mate non-randomly based on preference for certain trait values (Darwin 1859; 1871). Traditionally, these have been thought of as two distinct processes, with research directed primarily at understanding their phenotypic effects in terms of the evolution of traits that improve access to females, such as weaponry used in male-male combat, and exaggerated ornaments and behavioural displays, respectively (Darwin 1871). However, there remains a need to more fully characterize how these two components act together to determine the total variance in male sexual fitness, and also how they combine to affect phenotypic evolution. Additionally, increased attention has recently been given to the population genetic consequences of sexual selection, particularly with respect to its effects on mutation load. How the individual components of intersexual selection may contribute to this latter process is not well understood.

As gaining access to females is an essential component of male reproductive success, males of many species engage in direct, contest competition with other males to acquire and defend resources that are required by females (Emlen and Oring 1977; Andersson 1994). Variation in success at this stage of reproduction is known to contribute importantly to variation in mating success in some species, and territorial behaviour has thus been well studied with regard to the evolution of traits such as weaponry and other status signals (Moller 1987; Emlen and Nijhout 2000). As a result of the significant investment of time and energy that is generally required (Grantner and Taborsky 1998; Kelly 2008), a male's territorial success is likely to be strongly predicted by his condition, which can be defined as "the relative capacity to maintain optimal functionality of vital systems within the body" (Hill 2011). Variation in this component of mating success may therefore be expected to favour males in relatively high condition, with condition itself affected by both an individual's genetic quality and the environment they have experienced (e.g., resource availability). Importantly, the consequences of sexual selection arising from territorial competition for population mean fitness have not to my knowledge been examined.

This interest in understanding the population genetic consequences of sexual selection, particularly in relation to its role in reducing mutation load, has seen relatively recent renewal (e.g., Whitlock 2000; Agrawal 2001, reviewed in Whitlock and Agrawal 2009). While natural selection is expected to lead to adaptive responses, sexual selection has by contrast often been thought of as acting to reduce population mean fitness as a result of the evolution of costly sexual displays and preferences for these displays (Lande 1980), in addition to its role in generating sexual conflict (Pizzari and Snook 2003; Arnqvist and Rowe 2005; Pischedda and Chippindale 2006). However, provided that individuals with reduced non-sexual fitness also have reduced reproductive success (i.e. sexual fitness), sexual selection may act to reinforce natural selection, potentially promoting adaptation and increasing population mean fitness (Whitlock 2000; Lorch et al. 2003; Whitlock and Agrawal 2009). A reduction in the frequency of particular segregating deleterious alleles as a result of sexual selection has indeed been demonstrated in several recent studies (Radwan 2004; Hollis et al. 2009; Sharp and Agrawal 2008), although others have revealed little effect or have even suggested a cost (Holland and Rice 1999; Hollis and Houle 2011; Arbuthnott and Rundle 2012). What remains less well understood is how the individual components of sexual selection may contribute to this role, and one goal of this study was to address this question as it relates to territoriality as a component of male sexual fitness.

The second, related issue I aim to address here concerns how male-male competition and female mate choice contribute separately to the evolution of traits under selection. Although the two mechanisms have traditionally been thought of as being distinct from one another, studies that have considered these processes together have frequently assumed them to work in concert, with female choice facilitated by competition among males (Andersson 1994). This assumption likely relates to the expectation that competitive success is always indicative of a higher quality male, with females thereby able to maximize their fitness by choosing such males for mating (Andersson 1994; Berglund et al. 1996). The result is that traits reflective of dominance, such as large body size and weaponry, are therefore also expected to be used as signals in female mate choice (Berglund et al. 1996; Qvarnstrom and Forsgren 1998). Recently, however, a growing number of studies have suggested that male-male competition and female mate choice may in fact be counteracting, with dominant males not always being those preferred by females (Moore and Moore 1999; Casalini et al. 2009).

Particularly for species in which males compete over territories, competition may act either to facilitate female choice by excluding inferior males, or to hamper choice by excluding males that females might otherwise prefer (Wong and Candolin 2005). This latter observation is based on the fact that male dominance does not necessarily predict better genes or high parental quality, and the costs associated with mating with more dominant males may outweigh any potential benefits (Qvarnstrom and Forsgren 1998). Such a situation may be particularly likely to occur when the interests of females and males diverge, leading to an evolutionary conflict between the sexes (i.e. sexual conflict; Moore et al. 2001; Arnqvist and Rowe 2005). As the selection imposed by each component may therefore frequently differ in strength and form, with important consequences for trait evolution as well as fundamental questions such as the maintenance of genetic variation, the relative contribution of each should be quantified to generate a more complete understanding of how sexually selected traits evolve (Qvarnstrom and Forsgren 1998; Hunt et al. 2009).

Here, I used a laboratory population of the Australian fruit fly *Drosophila serrata* to examine territoriality as a component of male sexual fitness. Although not yet documented in *D. serrata,* territoriality has previously been demonstrated for both *D. melanogaster* and *D. simulans* males (Jacobs 1960; Dow and von Schilcher 1975; Hoffmann 1987) in addition to the endemic Hawaiian lekking species (Spieth 1974; Boake 1989) and can be observed and quantified in the laboratory. I was therefore able to test whether territoriality occurs among *D. serrata* males, to determine whether success in territory defense is condition-dependent, and to assess whether selection against low condition males is stronger given the inclusion of this component of male mating success. In addition, female mate choice has been relatively well characterized for this species, and is known to be based in part on a suite of long chain cuticular hydrocarbons (CHCs) that act as contact pheromones (Chenoweth and Blows 2003, 2005; Howard et al. 2003; Rundle et al. 2009; Delcourt et al. 2010; Sztepanacz and Rundle 2012). Past studies have also shown CHCs to be important to other aspects of communication in this (Petfield et al. 2005) and other *Drosophila* species, including playing a role in male-male competition in *D. melanogaster* (Wang et al. 2011). The goal of this study was to examine territory defense in *D. serrata* to further understand both how variation in this component of mating success may affect population mean fitness, and also how separate components of sexual selection, male-male territorial competition and female mate choice, each contribute to the evolution of sexually selected traits in this species.

MATERIALS AND METHODS

Stock Population

A stock population of *D. serrata* was created in November 2003 by mixing six laboratory populations originally collected from different sites along the east coast of Australia (Rundle et al. 2006). This stock has since been maintained under constant conditions (25°C; 12L:12D photoperiod) at a large population size (16 half-pint stock bottles) on a standard yeast-agar laboratory media with discrete, non-overlapping generations. An additional *D. serrata* population fixed for a homozygous recessive mutation resulting in an orange-eyed phenotype was also used. This population was housed at a large population size (8 half-pint stock bottles) with discrete, non-overlapping generations under the same conditions as outlined above.

Occurrence of territoriality and consequences for male sexual fitness

Flies were collected upon emergence and separated by sex as virgins within 24 h using light CO_2 anesthesia. Males and females were housed separately at a density of 10 individuals per vial. Wild type and orange-eyed (mutant) males were aged 24 h, then marked by clipping a small piece from alternate wings (i.e. left wing for wild type, right wing for mutant). Individuals were aged two days further, and territorial interactions examined after aspirating pairs of males (one wild-type and one mutant) into each of 100 clear arenas (modified from Hoffmann 1987) constructed from two petri dishes (100 x 15 mm), one inverted over the other. A small hole (covered with tape) was made in the top dish to allow addition of the flies, and a food cup (diameter = 20 mm, height = 1.2 mm), containing 5 ml of standard medium and a small ball of yeast paste, was placed in the center of each arena. The bottom petri dish was lined with tap water-saturated filter paper to maintain a humid environment. Males were given 24 h to interact and territorial success was then scored via spot counts, taken at 30 min intervals (across 2.5 h total), in which the position of each male relative to the food cup was recorded (i.e. on/off). Using the same protocol, two additional series of observations were made after adding either single males or pairs of previously mated females to each of 20 experimental arenas respectively.

To assess whether variation in territory defense ability affects male mating success, pairs of three-day-old wild-type virgin males once again competed in each of 90 arenas as described above. Territorial success was recorded via spot counts every 30 min from 13:00 to 16:00, and again from 10:00 to 12:00 the following day. A single virgin female was then aspirated into each arena, followed by two 3 h mating observation periods from 13:00-16:00 on the same day, and 09:00-12:00 on the following morning. Observations involved continuously scanning all replicates such that each arena was checked approximately every five minutes. The identity of the successful male was recorded whenever a mating was observed, with each mating counted only once.

To test for territorial behaviour, I first assessed whether the probability of one male being on the food source depended on the status (i.e. on or off) of the other male. This was done using an overall chi-square test of independence for the position of each male relative to the food source (i.e. both males on, both males off, wild type only on, orange-eyed only on), combining the data from each observation period. To eliminate issues associated with non-independence of counts from the same replicate arena at different observation periods, the observed chi-square value was compared to a null distribution of expected values generated from 10,000 randomizations. In each iteration of the randomization procedure, the occupancy status of one male within a particular replicate was randomly reassigned across all observation periods of that replicate, independent of the status of the second male but preserving the number of times it was observed on or off the resource (i.e. the occupancy rate). This method was similarly used to assess independence in the assays using two females. Standard chi-square tests of independence were subsequently done for each observation period separately, with Fisher's exact tests used for the assay with females due to a lower sample size.

Consistency across observations in the identity of the male that held the territory (i.e. whether a male in possession of the food resource tended to remain so) was assessed by counting the number of times that ownership switched between observation periods. A male was inferred as possessing the food resource when he was the sole occupant, and a switch was deemed to have occurred if the possessing male became the excluded male in a subsequent count. Observations in which neither or both males were on the food were considered non-informative and were ignored. The observed mean number of switches was compared to a null distribution of the expected number of switches generated from 10,000 randomizations. In each iteration of the randomization procedure, the status score of the two males (i.e. both on, both off, wild type only on, orange-eyed only on) were shuffled among replicates separately for each observation period. This procedure removed any correspondence across observation periods within a replicate (i.e. any consistency) while maintaining both the marginal and total occupancy rates.

To examine the relationship between territorial success and mating success, I first calculated territoriality difference scores for each male pair (i.e. the score of the male that most frequently occupied the resource minus the score of the other male). Spearman's rho correlation was then used to examine the relationship between this difference in territorial success and the difference in the number of matings obtained by the two males (i.e. number obtained by the male with the higher territoriality score minus the number obtained by the other male).

Condition manipulation and territorial competition trials

I attempted to generate adult males of varying condition via both environmental and genetic manipulations. The environmental manipulation consisted of two treatments that differed in larval rearing density. Treatments were established by allowing either 10 or 40 mated females (low and high density, respectively) to oviposit onto 10 mL of standard yeast media for 24 h. Vials (10 replicates per treatment) were checked daily, beginning at day 10, so that the number of emerging flies could be counted. On day 12, ten males from each replicate were collected, dried at 65 °C for 24 h, and weighed to the nearest 10^{-6} g.

The genetic manipulation of condition was conducted via full-sibling inbreeding. Virgin male-female pairs were established in each of 285 vials, and from the offspring of each a single male and female sibling pair was collected and transferred to new vials for egg-laying. This was repeated for two subsequent generations, resulting in a total of three generations of inbreeding (inbreeding coefficient, $f = 0.5$). At the F2 generation, a second male and female pair was also collected from each vial and mixed among vials to generate outbred control lines. This step was repeated at the F3 generation. Due to the loss of some lines at each generation, 90 inbred and 90 outbred ‘lines’ were used in the subsequent competition trials.

Flies were collected upon emergence and separated by sex as virgins within 24 h using light CO_2 anesthesia. Males and females were housed separately at a density of 5-7 per vial. To discriminate between competing males, high/low condition flies had a small piece clipped from their left/right wing respectively and were allowed to recover for two days. Trials were conducted over three days, and all males in a given experiment were of the same age (five days in the environmental manipulation, four days in the genetic manipulation).

To manipulate the opportunity for a male’s territory defense ability to contribute to his mating success, experimental arenas contained either a single resource located in the center, or two resources placed opposite one another. In both cases the resource consisted of a food cup containing 5 ml of standard medium and a ball of yeast paste (diameter of approximately 5 mm) as used in the previously described assays. Male pairs (one low condition, one high condition) were aspirated into each of 200 arenas for the environmental manipulation trials, and each of 180 arenas for the genetic manipulation. For both experiments, half of the arenas contained a single resource (i.e. high opportunity for competition over territories) and the other half two resources (i.e. low opportunity for competition over territories). After approximately 16 h, males were scored for territorial success by recording the position of each relative to the food source(s). Scores were taken every 0.5 h to a total of six counts.

After territorial scores were recorded, a single virgin female was aspirated into each arena and mating observations were made by scanning the arenas at approximately 5 min intervals.

The identity of the successful male was recorded whenever a mating was observed, with each mating counted only once. Observations were made for 3 h (13:00-16:00) immediately after the females were added, and continued the following morning for second 3 h period (09:00-12:00). Both males from each arena were then frozen for storage, dried at 65 °C for 24 h, and weighed to the nearest 10^{-6} g.

For the environmental and genetic manipulations, mean mass and time to emergence were compared between treatments using two-sample *t*-tests. The difference in territory and mating success between male pairs was calculated by subtracting scores of low condition/inbred males by those of high condition/outbred control males. One-sample *t*-tests were used to assess whether this difference score was significantly different from zero, with a positive score indicative of an advantage for high condition males. Two-sample *t*-tests were used to determine whether these difference scores differed between the two arena types (i.e. one vs. two food resources) representing low vs. high opportunity for territoriality.

Selection analyses

To estimate selection on CHCs and body size resulting from male-male territoriality and female mate choice, treatments were established that allowed successful and unsuccessful males from each of these two components to be identified. First, to identify males that were successful or unsuccessful in defending a territory (“winners” and “losers”, respectively), two day old virgin male pairs were marked by wing clipping, allowed to recover for two days, and then aspirated into each of 300 experimental arenas. Arenas were identical to those used previously, with the exception that the yeast paste in the center of the food resource was covered with a wire mesh to prevent access by males. This design allowed males to detect the food resource so as to encourage territorial defense, but prevented them from consuming the live yeast as this may affect CHC expression (A. White, unpublished data; Gosden and Chenoweth 2011). Eighteen hours after being introduced, pairs were scored for territorial success via six spot counts made every 0.5 h in which the location of each male was recorded (i.e. on or off the food resource).

To estimate selection on CHCs and mass resulting from female mate choice, 300 binomial female-choice mating trials were conducted by introducing two males to a vial containing a single virgin female. Vials were observed continuously and the chosen and rejected males were identified from the first mating to occur. To minimize potential effects of prior experience, the two males in a particular replicate were taken from separate housing vials, and only replicates in which a first mating was observed within 1 h were included in the analysis.

For each treatment (i.e. territorial competition and female choice), the losing (or rejected) male as well as the winning (or chosen) male was isolated for CHC extraction. Individual flies were treated as independent replicates, as past work has shown this to have no effect on the significance of the selection gradients (Rundle et al. 2005). Flies were anaesthetized under light CO_2 and individually added to 100 µl of hexane for 3 min followed by 1 min of agitation in a vortex mixer. Flies were then removed from the hexane vials, frozen at -20 °C, dried at 65 °C for 24 h, and weighed to the nearest 10^{-6} g. To minimize any confounding effects of temporal changes in CHCs on assay type (i.e. male-male territoriality vs. female choice), extractions of males from each treatment (territorial competition and female mate choice) were alternated throughout the day.

For each male isolated from the competition and mating trials, nine CHCs were analyzed using gas chromatography: (Z,Z)-5-9-$C_{24:2}$, (Z,Z)-5,9-$C_{25:2}$, (Z)-9-$C_{25:1}$, (Z)-9-$C_{26:1}$, 2-Me-C_{26}, (Z,Z)-5,9-$C_{27:2}$, 2-Me-C_{28}, (Z,Z)-5,9-$C_{29:2}$, and 2-Me-C_{30} (Howard et al. 2003). The areas under each of the nine chromatograph peaks were integrated and relative amounts were determined by dividing the area under each peak by the total area under all nine peaks. This corrects for technical error associated with their quantification and is less prone to experimental error than the use of internal standards (Blows and Allan 1998; Savarit and Ferveur 2002). To break the unit-sum constraint characteristic of such compositional data, proportions were transformed into eight logcontrast values for statistical analysis, using the proportional area under (Z,Z)-5-9-$C_{24:2}$ as the common divisor (Blows and Allan 1998).

Three multivariate outliers were identified and removed from the CHC/body size data using the Mahalanobis distance technique implemented in JMP (version 10.0; SAS Institute Inc., Cary, NC). Standardized sexual selection gradients (*β;* Lande and Arnold 1983) on the eight logcontrast CHCs and body size were estimated separately for each treatment (i.e. territoriality and female choice) using standard least squares multiple regression of relative success against standardized (mean = 0, standard deviation = 1) trait scores (Lande and Arnold 1983). Although fit via least squares, significance was determined using logistic multiple regression, fit via restricted maximum likelihood, because success scores are binomially distributed (Fairbairn and Preziosi 1996; Rundle et al. 2009). To determine whether selection on these traits differed between trial type (i.e. territoriality vs. female choice treatments), data were combined in a single analysis using the multiple regression model described above, but with the inclusion of a fixed effect term representing the main effect of trial type (male-male territoriality vs. female choice), along with nine additional terms representing the interaction of each trait with trial type. A likelihood ratio test was then used to compare the fit of this full model with a reduced one lacking the nine interaction terms.

RESULTS

Occurrence of territoriality

Significant non-independence in the probability of occupying the food resource was detected between the two males overall ($\chi^2 = 73.80$; randomization procedure, $P < 0.0001$; Fig. 1). This non-independence was also highly significant in separate tests of each observation period (Table 1). In all cases, this involved a substantial overrepresentation of the situation in which one male was on the food and the other male was off (and hence that far fewer pairs than expected were either both on or both off of the food source). When placed singly into arenas, males were found on the resource in 70.8 ± 18.7% (mean ± SD) of the observations, whereas in trials with pairs of males, both were found together on the resource in only 7.7 ± 1.5% (mean ± SD) of the observations. In contrast to results for males, occupancy of the food resource by one female was independent of the location of the other female, both overall ($\chi^2 = 1.07$; randomization procedure, $P = 0.710$) and in separate tests of each observation period (Table 2). Furthermore, both females were found together on the food source in 68.8 ± 10.3% (mean ± SD) of the observations (Fig. 2).

When territory ownership was examined across the five observation periods, I found an average of only 0.283 switches to have occurred in the identity of the male occupying the food source. This is significantly less than expectation (mean = 1.166; randomization procedure, $P < 0.0001$; Fig. 3), indicating strong consistency in which male possessed the resource. Similar results were obtained via an alternative analysis in which replicates were differentially weighted based on their occupancy rates (unpublished results). Furthermore, consistent with territorial defense being a component of male sexual fitness, there was a positive association between territorial success and subsequent mating success (Spearman's rho = 0.232, $P = 0.036$; Fig. 4).

Condition dependence

The manipulation of larval rearing density produced significant differences in both emergence time and mass (Fig. 5), suggesting that this treatment affected adult condition. In particular, flies raised under low density emerged nearly two days earlier and weighed almost 50% more on average than flies reared in the high density treatment (emergence time: $t_{18} = 9.48$, $P < 0.001$; mass: $t_{198} = 26.45$, $P < 0.001$).

In the presence of a single food resource (i.e. high competition for territories), high condition males were significantly more successful than low condition males in acquiring a territory (one-sample *t*-test: $t_{99} = 2.63$, $P = 0.010$). By contrast, this advantage of high over low condition males was absent in the arenas with two food resources and hence reduced competition for territories (one-sample *t*-test: $t_{99} = 0.87$, $P = 0.387$; Fig. 6a).

In addition to having higher territorial success, high condition males also acquired a higher number of matings than low condition males when competition for territories was high (i.e. in the presence of a single food resource; one-sample *t*-test: $t_{99} = 3.20$, $P = 0.002$; Fig. 6b). High condition males also tended to have a mating advantage when competition for territories was low (i.e. two food resources), suggesting that mating success itself may be condition-dependent independent of territorial success. However, this advantage was reduced compared to the single territory treatment and was marginally non-significant (one-sample *t*-test: $t_{99} = 1.93$, $P = 0.056$; Fig. 6b). That the mating advantage of high over low condition males was reduced and no longer significant when competition for territories was low is consistent with territoriality being a key component of mating success, although this difference in mating advantage between competition treatments was not significant (two-sample *t*-test: $t_{198} = 0.57$, $P = 0.569$).

Inbred males were significantly smaller than outbred controls (mean ± SD; outbred: 0.282 ± 0.024 mg; inbred: 0.268 ± 0.027 mg; $t_{335} = 4.90$, $P < 0.001$), although this difference in mass (~5%) was much smaller than that observed in the previous larval density manipulation (~50%). Also in contrast to results from the density manipulation, outbred control males showed no significant advantage in either territorial success (one-sample *t*-test: $t_{89} = 0.052$, $P = 0.959$) or mating success (one-sample *t*-test: $t_{89} = 1.305$, $P = 0.195$) compared to inbred males in the single territory treatment. Outbred males similarly had no advantage in either measure when multiple territories were present (one-sample *t*-tests, territorial success: $t_{89} = 1.687$, $P = 0.095$; mating success: $t_{89} = 1.321$, $P = 0.190$), and the difference between treatments was also not significant for territorial success (two-sample *t*-test: $t_{156.82} = 0.992$, $P = 0.323$) or mating success (two-sample *t*-test: $t_{178} = 0.124$, $P = 0.902$).

Selection on CHCs and body size

Sexual selection on male CHCs and mass differed significantly between the territoriality and mate choice trials (Likelihood ratio test, $\chi^2 = 20.66$, $P = 0.014$). In the territoriality trials, success was positively associated with increasing male body size but not with variation in any of the logcontrast CHCs (Table 3). The reverse was true in the mate choice trials, with a significant effect of two logcontrast CHCs and no effect of body size (Table 3).

DISCUSSION

Despite a large body of research in the area of sexual selection, we currently lack an integrated understanding of how selection arising from the individual components of male mating success combine to affect trait evolution and population mean fitness. In this thesis, I used a laboratory population of *D. serrata,* a model system for the study of female preference for male sexual display pheromones, to examine how male territorial competition contributes to these outstanding issues. First, I demonstrated the occurrence of territoriality in males of this species, and also that variation in territorial success is positively correlated with mating success. Secondly, I conducted environmental and genetic manipulations to examine whether territorial success is condition-dependent, and how this subsequently affects variation in mating success. Results from the environmental manipulation indicate that high condition males had increased territorial and mating success, and furthermore that this advantage in mating success tended to be greater given a high opportunity for territory defense. No effects of the genetic manipulation were detected, although male body size, a trait later shown to be correlated with territorial success, was approximately an order of magnitude smaller than in the diet manipulation.

Lastly, I investigated how male-male territorial competition contributes to sexual selection on CHCs and body size in males. Previous studies have shown CHCs to be under significant directional selection arising from female choice in this species (Chenoweth and Blows 2003, 2005; Howard et al. 2003; Rundle et al. 2009; Delcourt et al. 2010; Sztepanacz and Rundle 2012), and my results are consistent with this. Although territorial success by contrast did not generate directional selection on any of eight logcontrast CHCs examined, there was significant directional selection on body mass arising from this component of male sexual fitness. The implications of these results are discussed below.

Territoriality as a component of male sexual fitness in D. serrata

As expected, males showed evidence of behaviour consistent with the occurrence of territory defense. Given an environment with a discrete food resource, single males occupied this resource at the exclusion of other males and the identity of the territory owner also tended to remain consistent over time. While aggressive male-male interactions have been described previously in *D. serrata* (Hoikkala et al. 2000), this study is the first to my knowledge to present evidence of territorial defense in this species. This finding is consistent with what is known for both *D. melanogaster* and *D. simulans* (Jacobs 1960; Dow and von Schilcher 1975; Hoffmann 1987) as well as the Hawaiian lek species (Spieth 1974; Boake 1989).

In contrast with the patterns observed in males, female pairs showed no evidence of territorial behaviour despite being highly attracted to the food resource. This suggests that fitness gains for male territory holders are related to the greater availability of potentially receptive females for mating. Consistent with this expectation, variation in the ability of males to defend a territory was correlated with subsequent mating success. Similar relationships have likewise been demonstrated in *D. melanogaster* (Dow and von Schilcher 1975; Hoffmann and Cacoyianni 1989), and may relate to the function of the defended resource as a site for oviposition and/or to its importance for female fecundity, which has been shown to increase in proportion to the availability of live yeast (Linder and Rice 2005). Territorially successful males do not always have a mating advantage, however, and in *D. melanogaster* the relationship between territorial success and mating success has been shown to differ depending on the competitive environment, whether females are virgin or mated, and among different populations and genotypes (Hoffmann and Cacoyianni 1989; Cabral et al. 2008). Such issues remain to be addressed in *D. serrata*.

While *D. serrata* behaviour has not been well studied under natural conditions, field observations for *D. melanogaster* suggest that the defense of discrete resources by males may be common (Partridge et al. 1987; Taylor and Kekic 1988; Hoffmann and Cacoyianni 1990). Furthermore, females tend to use areas of decaying fruit, which are often ephemeral and unevenly distributed, as food and a substrate for oviposition (Atkinson and Shorrocks 1981; Hoffmann 1987). Consistent with what was found in this laboratory population, territoriality may therefore also contribute importantly to variation in reproductive success under natural conditions. The relative contribution of territoriality and mating success to the total variance in male sexual fitness, particularly in the wild, remains a question for future study.

Condition-dependence

Results from the larval density manipulation showed that high condition males were more successful in defending the food resource, indicating that territorial defense is condition-dependent. Furthermore, sexual selection acting against relatively low condition males tended to be stronger when the opportunity for territory defense to contribute to male mating success was included. Selection arising from variation in territory defense as a component of male mating success may therefore be an important mechanism by which sexual selection can act to reinforce natural selection, thus helping to improve population mean fitness.

Other studies that have similarly partitioned total sexual selection to examine how individual components may contribute to this process have reported results in line with what was found here, with sexual selection acting to improve population mean fitness. For example, search effort (MacLellan et al. 2009) and offensive sperm competition (Clark et al. 2012) have each been found to contribute to selection against deleterious alleles and low condition males, respectively. However, in contrast to these results as well as those reported by certain studies that have looked at total sexual selection against particular segregating deleterious alleles (e.g., Radwan 2004; Sharp and Agrawal 2008; Hollis et al. 2009), other studies have revealed little effect on population mean fitness or have even suggested a cost (Holland and Rice 1999; Hollis and Houle 2011; Arbuthnott and Rundle 2012). Future work should examine how the various components of male mating success combine to affect the strength of sexual selection against deleterious mutations, particularly with respect to natural populations.

The finding of a non-significant difference in the mating advantage of high condition males between treatments, despite being greater given the inclusion of territory defense, may be in part attributable to the fact that high condition males, which here weighed ~50% more on average than males in low condition, are expected to perform better overall regardless of the competitive environment. It is therefore not unexpected that high condition males would have also achieved a greater number of matings in the two territory treatment, in which the opportunity for territoriality to contribute to male mating success was limited. That the mating advantage for high condition males did tend to be greater given the inclusion of territory defense, however, indicates that this component of male sexual fitness likely contributes at least in part to selection against low condition males. Future research should be aimed at further assessing the strength of this conclusion.

Additionally, the lack of an effect on territorial or mating success as a result of the genetic manipulation, together with the comparatively low difference in mass (~5%) between males from outbred and inbred lines, suggests that this manipulation may not have generated significant variation in condition. If territorial success is largely determined by the size of competing males, the small difference in mass created by three generations of inbreeding may not have been sufficient as to significantly affect competitive outcomes. Alternatively, condition is a multifaceted trait and it is possible that the environmental manipulation simply affected different aspects as compared to the genetic manipulation. Although environmental manipulations, including diet and density, have frequently been used as a proxy for deleterious alleles carried by an individual (e.g. Bonduriansky and Rowe 2005; Clark et al. 2012), the link to genetic effects has yet to be well established (Whitlock and Agrawal 2009). Similar to what was found here, for example, Clark et al. (2012) showed that while high condition males generated via a diet quality manipulation had an advantage in offensive sperm competition, no such effect was detected when condition was manipulated by introducing one of several individual mutations with known deleterious effects on non-sexual fitness. The extent to which environmental manipulations can serve as a useful substitute for genetic effects remains an important area for future study.

Selection arising from male-male competition and female choice

Empirical studies of sexual selection have often focused on either female choice or male competition alone, or have studied total selection without attempting to understand how each mechanism contributes separately to the evolution of traits under selection (Hunt et al. 2009). Here, I investigated the roles of male-male competition via territoriality, and female mate choice, in generating selection on CHCs and body size in *D. serrata.* As demonstrated in previous studies of this (Delcourt et al. 2010, Sztepanacz and Rundle 2012) and other populations of *D. serrata* (Hine et al. 2004, 2011; Chenoweth and Blows 2005), I found CHCs to be under significant directional selection arising from female choice. Male competition over territories, by contrast, generated no directional selection on any of eight logcontrast CHCs but did result in significant directional selection on body mass.

Despite this lack of direct evidence for a role of CHCs in territory defense by *D. serrata* males, territoriality could still alter selection on CHCs via its effects on female mate choice. If competition over territories and female choice occur sequentially in *D. serrata,* then the former could limit the pool of males available in the latter. Results here showed that territorial contests tended to favor heavier males, thus acting to remove the smallest males from the pool of individuals available for females to select from. Scoring males for the vector of directional selection gradients on CHCs ($\boldsymbol{\beta}$) to generate a single trait (CHCβ) representing the multivariate combination of CHCs that best determines mating success (see McGuigan et al. 2011), I found a weak but positive phenotypic correlation between mass and multivariate attractiveness that approached significance (CHCβ; $r = 0.082$, $P = 0.066$). These results together suggest that selection here might be reinforcing; male competition not only acted to remove the smallest males, but also tended to restrict female choice to those males that displayed comparatively more attractive CHC blends.

While the role of CHCs in mate recognition and courtship have been well characterized in *D. serrata* (e.g., Blows and Allen 1998; Howard et al. 2003; Rundle et al. 2005), the role that these signals might have in regulating male-male aggression remains poorly understood.

Results here indicate that variation in the relative concentration of eight CHCs known to be important to female mate choice does not contribute to variation in territorial success. Pheromones have been found to stimulate aggressive behaviour in a variety of both invertebrate and vertebrate species (Chamero et al. 2007), however, and more specifically the olfactory pheromone 11-cis-vaccenyl acetate (cVA) as well as several CHCs have been identified in the regulation of aggression in *D. melanogaster* (Wang and Anderson 2010). Also in *D. melanogaster,* masculinization of female pheromones has been shown to cause males to stop courtship and instead behave aggressively, further indicating that pheromonal cues are used by *D. melanogaster* males in recognizing conspecifics as competitors (Fernández et al. 2010). The blend of CHCs produced by all *Drosophila* species generally consists of ~20 compounds, and intersexual variation in their relative concentrations is generally observed (Ferveur 2005). While it is then possible that pheromones other than those examined here do have some role in regulating aggression in *D. serrata,* the present study was aimed solely at examining selection on eight CHCs known to be important to female choice in this species.

While results here indicate that male size and composition of CHCs separately influenced the outcome of male-male territorial competition and female mate choice, respectively, males of many species are likely to display traits that do influence the outcome of both mechanisms (Berglund et al. 1996). The degree and form with which the two mechanisms are likely to interact may vary significantly between species and environmental contexts, and is likely to be heavily influenced by factors such as the presence of dominance hierarchies, the degree of sexual conflict, and whether the mechanisms occur simultaneously or sequentially (Moore et al. 2001; Hunt et al. 2009). It therefore remains important to examine the various components of both inter- and intrasexual selection if we are to fully characterize how sexual selection may be operating in a given system.

Chapter 3

Future Directions

An outstanding issue in sexual selection research concerns the relative importance of the various components of male mating success (e.g., search effort, resource defense, sperm competition) to explaining the total variance in male sexual fitness, particularly with respect to natural populations. If a single trait contributes to the outcome of multiple components of mating success, some of which may occur sequentially, then each component may restrict the outcome of the next with important consequences for the strength and form of selection. Under such conditions, selection gradients estimated via assays involving a single component of sexual fitness will misrepresent total selection on the trait. The need therefore exists for more empirical studies that not only consider how sequential episodes of selection may be operating in isolation, but that also combine these episodes to examine total sexual selection acting on male sexual traits of interest (Hunt et al. 2009).

An additional, important question for future research is whether environmental manipulations of condition are good proxies for genetic effects. Discrepancies between environmental and genetic manipulations have been reported here and elsewhere (e.g., Clark et al. 2012), and the link between the two has yet to be well established (Whitlock and Agrawal 2009). Environmental manipulations may, for instance, alter different aspects of condition as compared to mutations, which can lead to contrasting effects for certain components of mating success (Clark et al. 2012). While other methods such as mutagenesis, the use of single mutations of large phenotypic effect, mutation accumulation studies, and inbreeding may be preferable, environmental manipulation is likely to be the most feasible option for a variety of study organisms. The use of multiple methods, both environmental and genetic, may help to further elucidate the link between condition, trait values, and the outcomes of various components of mating success. This will ultimately help in better understanding the connection between sexual selection and mutation load.

Table 1. Total number of males observed (expected) in each of four states relative to a yeast food resource in experimental arenas, as determined in five sequential observation periods.

Observation	Wild-type	Orange-eyed	Both	Neither	χ^2	*P*
1	27 (19.3)	37 (29.3)	9 (16.7)	26 (33.7)	10.478	0.001
2	38 (30.4)	26 (18.4)	9 (16.6)	26 (33.6)	10.281	0.001
3	41 (30.7)	29 (18.7)	8 (18.3)	21 (31.3)	18.362	< 0.001
4	41 (31.3)	27 (17.3)	6 (15.7)	25 (34.7)	17.033	< 0.001
5	41 (31.3)	27 (17.3)	6 (15.7)	25 (34.7)	17.033	< 0.001

Table 2. Total number of females observed (expected) in each of four states relative to a yeast food resource in experimental arenas, as determined in four sequential observation periods.

Observation	Female #1	Female #2	Both	Neither	P^a
1	6 (5.95)	2 (1.95)	11 (11.05)	1 (1.05)	1.000
2	3 (3.4)	2 (2.4)	14 (13.6)	1 (0.6)	0.509
3	1 (2.25)	3 (4.25)	14 (12.75)	2 (0.75)	0.140
4	1 (0.85)	3 (2.85)	16 (16.15)	0 (0.15)	1.000

[a] *As determined using Fisher's exact tests (two-sided).*

Table 3. Standardized linear selection gradients (β) on body mass and logcontrast cuticular hydrocarbons (CHCs) from assays of male-male territoriality and female mate choice.

	β	
	Male-male territoriality	Female choice
Body mass	0.152**	0.022
(Z,Z)-5,9-$C_{25:2}$	0.040	-0.103
(Z)-9-$C_{25:1}$	0.034	0.022
(Z)-9-$C_{26:1}$	-0.010	0.009
2-Me-C_{26}	-0.074	0.107
(Z,Z)-5,9-$C_{27:2}$	-0.038	-0.038
2-Me-C_{28}	0.020	-0.070
(Z,Z)-5,9-$C_{29:2}$	-0.002	0.108*
2-Me-C_{30}	-0.102	0.147*

* $P < 0.05$, ** $P < 0.01$

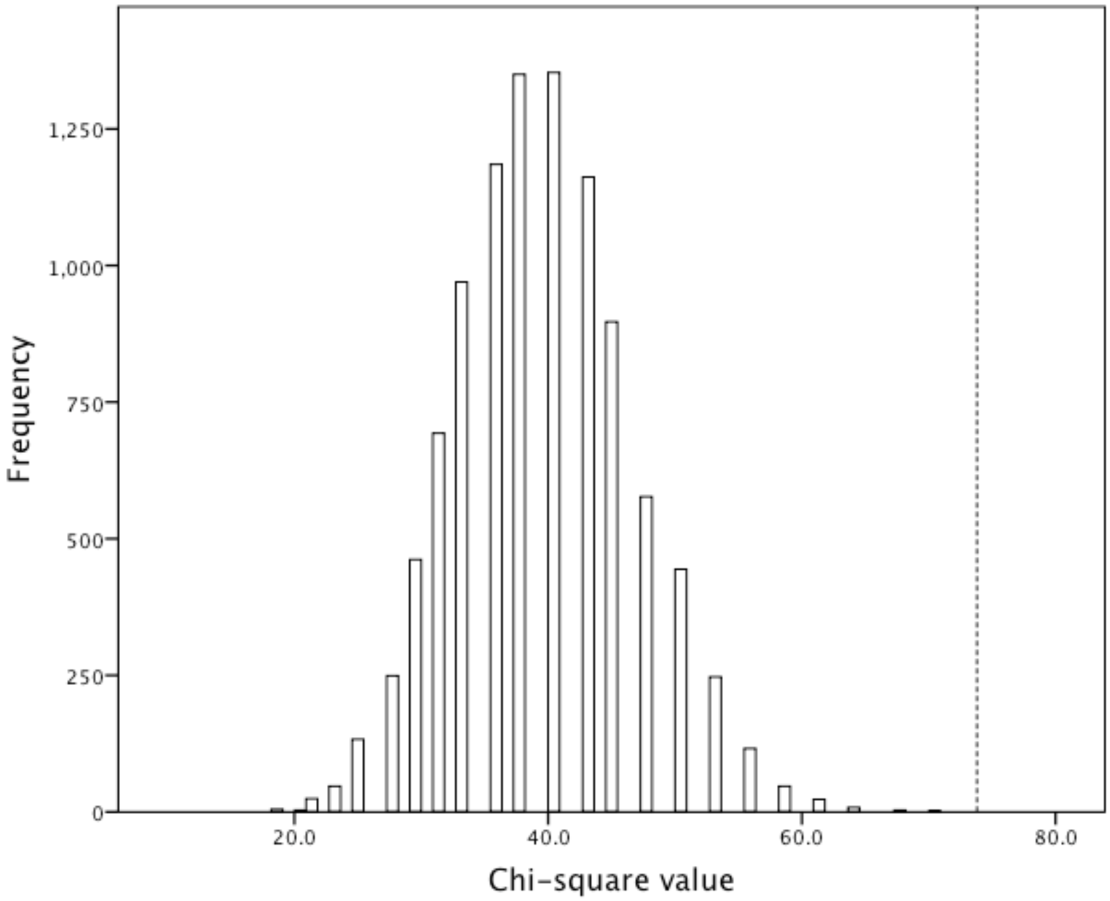

Figure 1. Observed χ^2 value (dashed line) testing for independence in the position of the two males in relation to the food source (i.e. both males on, both males off, wild type on, orange-eyed on), as compared to a null distribution of expected values generated from 10,000 randomizations.

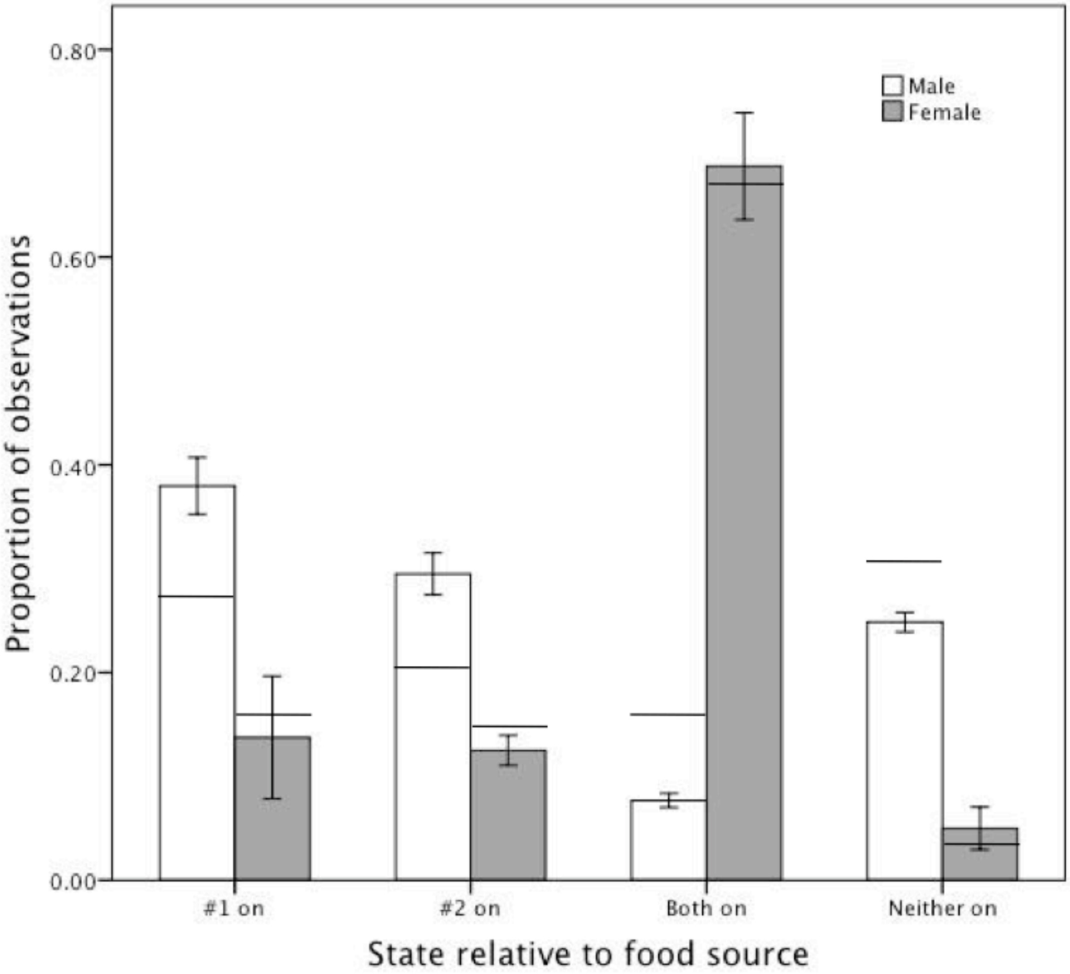

Figure 2. Average proportion of observations (± SE) in which pairs of males or females were in each of four states relative to a yeast food resource. Horizontal black lines indicate expected values for each category.

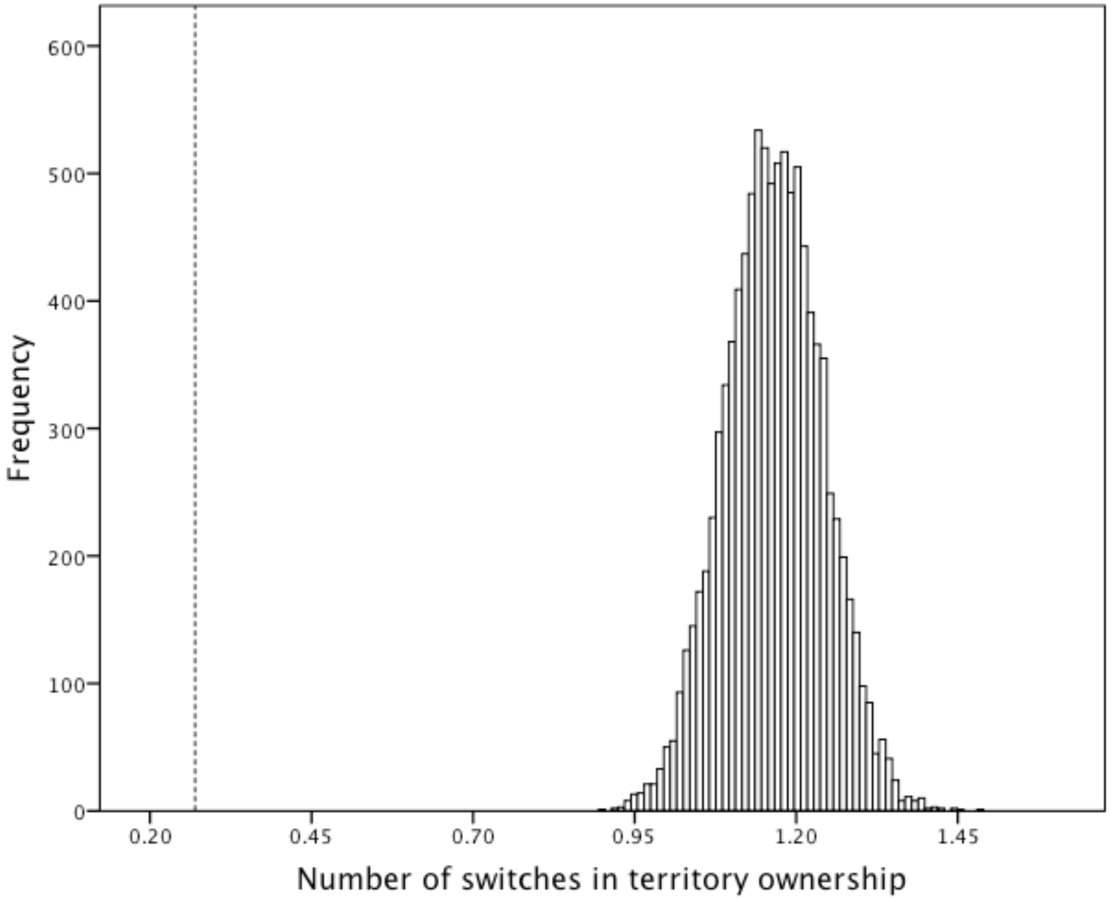

Figure 3. Frequency distribution of the expected number switches in territory ownership between male pairs, as generated from 10,000 randomizations assuming no correspondence in ownership across observation periods, and the observed value for the mean number of switches (dashed line).

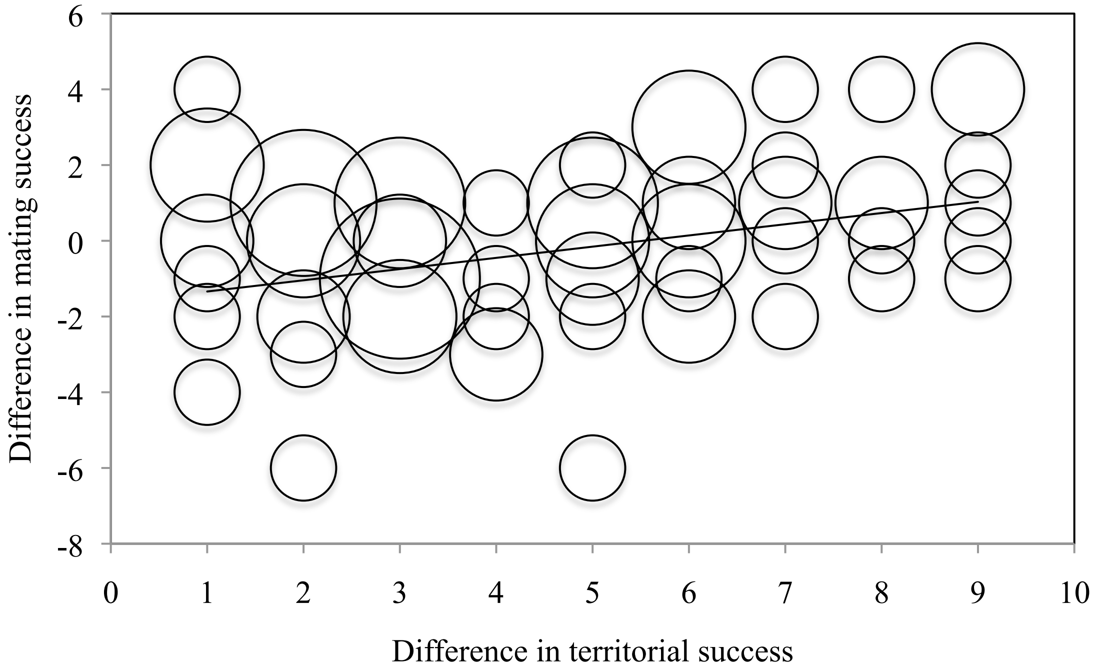

Figure 4. Variation in relative mating success (number of matings for the territory "winner" – number for the "loser") with relative territorial success (score "winner" – score "loser"; Spearman's rho = 0.232, *P* = 0.036). Circle areas are proportional to the number of contributing data points.

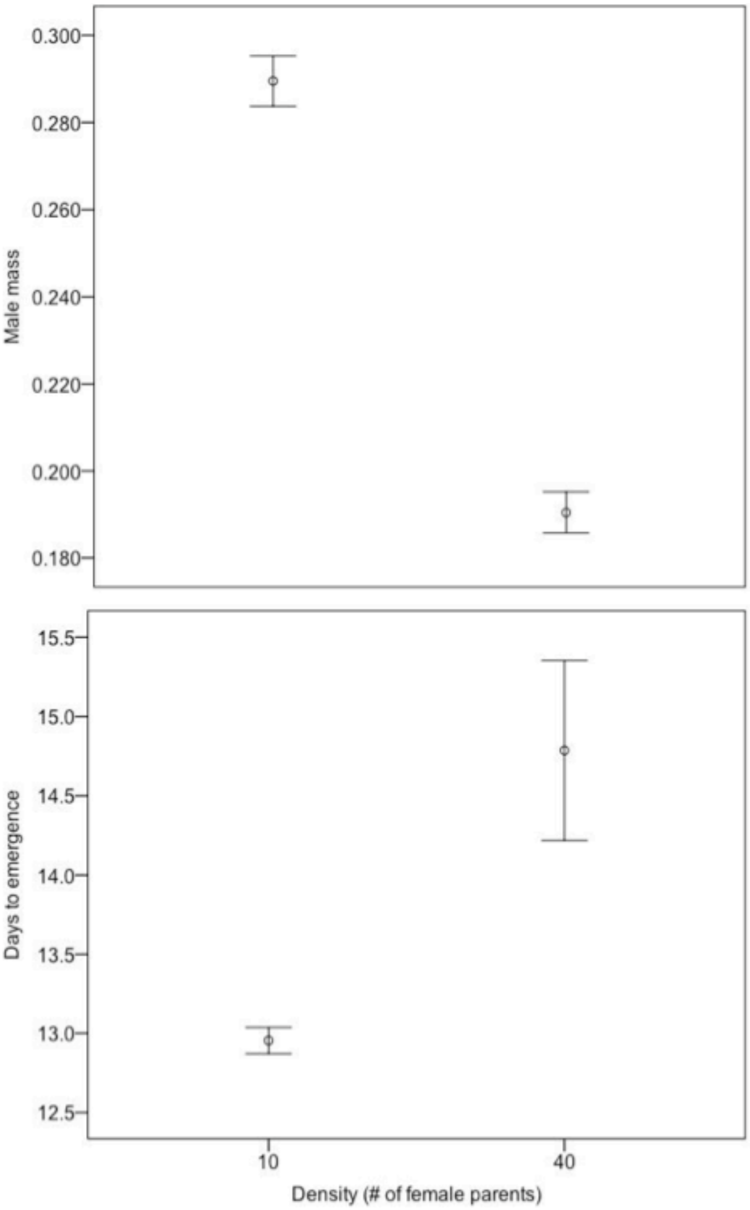

Figure 5. Average mass and emergence time (± 2 SE) for flies reared under low and high larval density conditions.

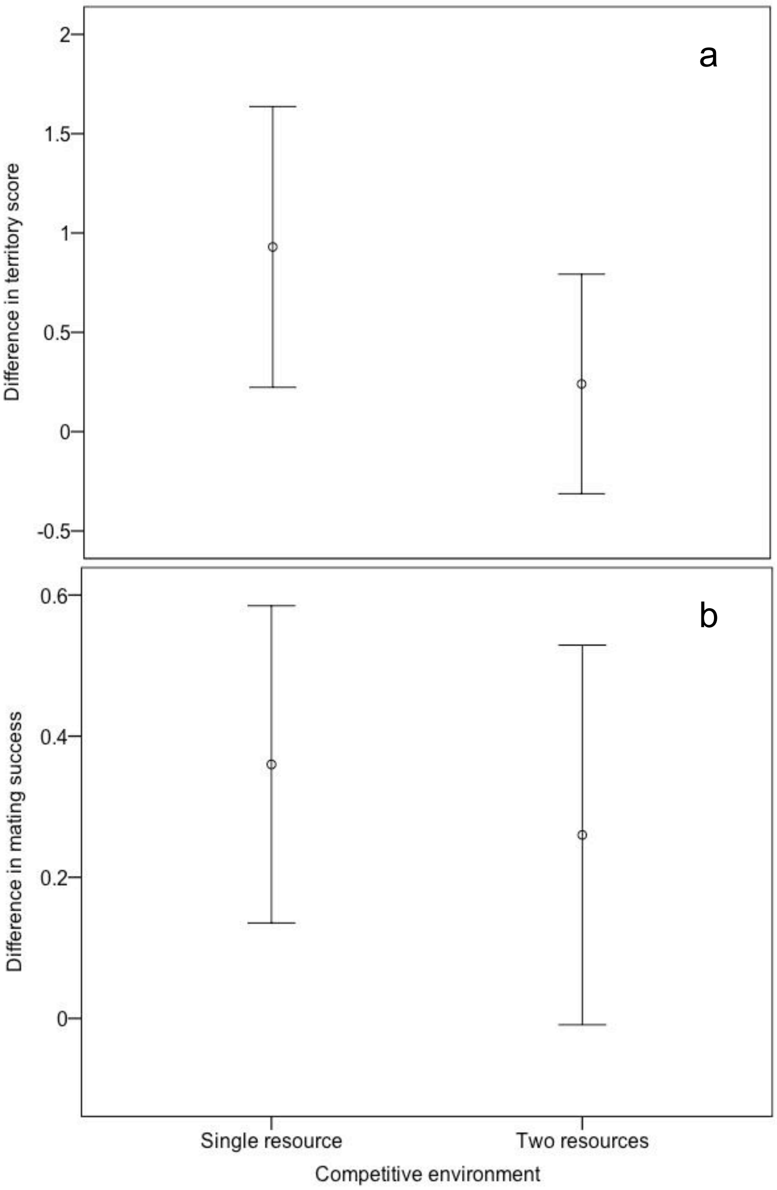

Figure 6. Average difference scores (high – low condition males, ± 2 SE) for **(a)** territorial success and **(b)** mating success in environments that contained either one or two food resources.

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