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I, __________________________ Alison Fay Cooperman ________________, hereby submit this work as part of the requirements for the degree of:

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in the Species *Drosophila bipinctinata*

This work and its defense approved by:

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Male Secondary Sexual Traits and Mating Behavior in the Species *Drosophila bipectinata* Duda (Diptera: Drosophilidae)

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ABSTRACT

The species *Drosophila bipectinata* Duda was used in a series of projects aimed at appending empirical support to vital aspects of sexual selection theory, including mechanisms of selection and ‘good genes’ models. The first chapter reports no evidence to support that male vigor, as measured by latency and duration of copulation, can explain the mating success of males larger in body and comb size found in a previous field study. Also, no evidence of female cryptic choice was found in this system. The second chapter reports a genetic association between two condition dependent secondary sexual traits; the finding of a positive association between two traits that differ in kind (sex combs vs. courtship song), and thus presumably have different developmental pathways, provides evidence of the presence of ‘good genes’ in this system. Future directions include testing for evidence of female choice for male *D. bipectinata* song and/or comb size.
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GENERAL INTRODUCTION

When Darwin published *The Descent of Man, and Selection in Relation to Sex* in 1871, he recognized that sexual selection is a distinct form of natural selection. Sexual selection was Darwin’s (1871) answer to three major questions: why are males and females usually so different, how can we explain exaggerated male sexual traits, and how can we explain the existence of characters that would seem detrimental to survival?

Natural selection is the idea that genes which produce characteristics that increase survival in a particular environment will be more abundant in the next generation, while sexual selection promotes the genes which produce characteristics that increase an organism’s success in mating and ensure that its gametes are successful in fertilization. The traits that are influenced by sexual selection function either in competition with members of the same sex (i.e., intrasexual selection) and/or in interactions with members of the opposite sex (i.e., intersexual selection), and can sometimes oppose natural selection.

The basis of many aspects of sexual selection is that the males and females of most species have conflicting strategies for maximizing the number of offspring harboring their genes (Andersson, 1994; Birkhead, 2000). In many species, the contribution of the male to the survival of the offspring is negligible, as reflected in the investment of a relatively small quantity of resources. Males often maximize their reproductive success by inseminating the eggs of as many females as possible, and/or preventing other males from inseminating the eggs of females that they have inseminated. In contrast, the female is usually more selective in choosing a male to mate with to ensure that her offspring are of high quality and that her resources are sufficient to secure their
viability. Since sexually mature females apportion a large amount of resources to generating eggs (and sometimes to caring for offspring) and thus produce relatively fewer gametes, females are normally sexually receptive for a short period. Thus, sexually active males typically outnumber sexually receptive females, so the competition for mates is more intense in males than in females. These general principles have several consequences:

1) The evolution of exaggerated sexual traits is normally restricted to males, the more competitive sex. The traits that advance male reproductive success are extremely diverse, and often extravagant: stags have antlers, male elephant seals gain a fighting advantage with size, various bird species have different kinds of feather displays, male bowerbirds build large and highly decorated nests to attract females, male dung flies wrestle with other males for the opportunity to copulate with females (reviewed in Andersson, 1994; Birkhead, 2000).

2) Darwin (1871) recognized that the characteristics that advance male reproductive success often increase the risk of death. The primary function of the antlers of stag deer is fighting other males for the opportunity to mate with does, and the long, ornate feathers of the peacock are a display to make males more attractive to peahens. However, both characteristics can incur fitness costs in other contexts: producing feathers and antlers uses biosynthetic resources, extravagant feathers attract predators, and fighting weakens stags. The conflict between sexual selection and natural selection is exemplified by the observation that male garden spiders die spontaneously, immediately after locking their genitals into the female’s genitals, a maneuver that blocks mating by other males (Foellmer and Fairbairn, 2003).
3) The evolution of male characteristics that attract females would not have reproductive advantages if the females did not find the male traits appealing (Birkhead, 2000; Wiens, 2001). Thus, the evolution of male reproductive traits is accompanied by the co-evolution of female preference for those traits. Co-evolution can lead to exaggeration of male traits, because the expression of the male trait and the female preference for that trait may become genetically linked. Greater expression is accompanied by greater preference, and the exaggeration of the traits continues until the costs become too great and natural selection imposes limits (Fisher, 1930; Lande, 1981; Kirkpatrick, 1982).

Many of these aspects of sexual selection can be (and have been) addressed empirically with phenotypic studies. The phenotypic approach is most appropriate in studying the targets of sexual selection, mating preferences, and the mechanisms of sexual selection. Similarly, the study of sexual selection can profit in many ways by the integration of quantitative genetic techniques. Knowledge of the extent of genetic variation and covariation of traits involved in sexual selection is essential to understanding the potential of, and constraints on, the evolution of these traits.

Secondary sexual traits, like other life-history traits, have high additive genetic variance compared to non-sexual traits (Houle, 1992; Pomiankowski and Møller, 1995). One explanation as to how high genetic variability is maintained in populations was offered by Rowe and Houle (1996). They argued that high genetic variability in sexual traits can be explained when we assume that they are condition-dependent, and that there is high genetic variance in condition.
Rowe and Houle (1996, pg. 1416) define condition as follows: “We imagine the life history as a process of accumulating resources that are then allocated to the production or maintenance of traits that enhance fitness. We will refer to the pool from which resources are allocated as condition…” During the transition of a trait from stabilizing natural selection to directional sexual selection, it is predicted that the trait will become more physiologically costly because with increasing exaggeration of the trait more resources are allocated to the trait at the expense of other fitness enhancing traits. The exaggeration will stop when the benefits of exaggeration are balanced by the costs. Once a trait becomes costly, it is expected to evolve condition dependence because individuals in higher condition are better able to pay higher costs of further exaggeration than those in lower condition. A crucial assumption in Rowe and Houle’s (1996) explanation is that there exists high genetic variance in condition.

The phenotypic expression of this potential variation in condition may help to explain the differential mating success of individuals exhibiting differences in sex trait expression, and support the ‘good genes’ models of sexual selection. A ‘good gene’ is defined as an allele that increases fitness independent of the genetic architecture of the remaining genome. When variation in fitness exists as a result of good genes, the population will respond to directional selection as their effects are additive (Neff and Pitcher, 2004). In the context of Rowe and Houle’s (1996) “genic capture” model, the good genes, then, may comprise the alleles responsible for resource acquisition. These ideas motivated the present work into a Drosophila system, the overall aims of which were to test whether variation in secondary sexual traits is indicative of individual quality (i.e., condition).
**Drosophila bipectinata** Duda, the organism chosen for a test of these hypotheses, is part of the *ananassae* species subgroup of the larger *D. melanogaster* group. *Drosophila bipectinata* is a widely distributed species ranging from India to Fiji and Samoa in the South Pacific. The substrate where most of the activity of the flies occurs is the decaying fleshy fruit of various plant species (Polak et al., 2004). Feeding, mating, oviposition, and maturation of pupae all take place on a single substrate—the fleshy fruit of random plant species. The mating system most closely resembles a scramble competition, where there is a premium on males who are able to locate and mate with multiple females within a narrow mating period, and thus male vigor would be a valuable fitness-enhancing attribute to males in this system (Thornhill and Alcock, 1983).

*Drosophila bipectinata* is one of 6 closely related species and subspecies in the *bipectinata* species complex, which also include *D. parabipectinata, D. malerkotliana, D. pseudoananassae, D. nigrens,* and *D. pallens* (Bock, 1971, 1978; Singh and Singh, 2001). The species in this complex, which are sympatric over most of their geographic range, differ mainly in two sexually dimorphic characters—pigmentation and sex comb morphology.

The sex comb of *Drosophila* is an array of modified bristles that develop at a precise position on the first pair of legs from a set of precursor bristles present in both sexes. Male *D. bipectinata*, the subject of our studies, lack pigmentation and so resemble females who similarly lack any abdominal pigmentation (Kopp and Barmina, 2005). Males of this species, however, differ from females in possessing rotated, obliquely-oriented sex combs.
The sex combs are a recent evolutionary novelty; most \textit{Drosophila} species do not have sex combs, although the precursor bristles are always present. Among the flies that do have them, the size and structure of the sex combs show dramatic variation, ranging from a pair of simple straight bristles to flies that have over 150 curved teeth. Male \textit{D. bipectinata} show combs positioned in three regions designated C1 (bearing 2-8 teeth per comb), C2 (bearing 4-11 teeth per comb) and C3 (bearing 0-4 teeth per comb) (Polak et al., 2004; Fig 1A: Chapter 2).

While the role of the sex combs has not been definitively determined, there is evidence that their function may differ between flies of the \textit{melanogaster} group in which there is one row of teeth and those of the \textit{obscura} group in which males have two. Spieth (1952) and Cook (1977) observed that in the \textit{melanogaster} group, the combs are used in grasping the female, specifically her ovipositor (Coyne, 1985), but in the \textit{obscura} group they may, in addition, assist males in spreading the female’s wings. No studies have addressed the evolutionary significance of intraspecific variation in the numbers of teeth in the sex combs (but see Kopp and Barmina, 2005).

Because the number of sex comb teeth may affect mating, the number of teeth and their positioning are likely targets of sexual selection. This selection should cause rapid changes in sex comb morphology and changes in mating behavior (Carson and Lander, 1984), although other possibilities, such as genetic drift, exist. Supporting the sexual selection views, sex combs differ between sister taxa in the number of teeth per row, in the number of rows, and in the positions and orientation of rows. In fact, taxonomic distinctions between the species in the \textit{bipectinata} complex can be assessed wholly on the differences in this trait (Bock, 1971; Crossley and Taylor, 1985, Kopp and Barmina,
Moreover, sexual selection for increased tooth number has been documented in nature; for example, Polak et al. (2004) showed that males harboring more teeth in the C2 region of the combs were more apt to be found mating.

Therefore, the intentions of this thesis are to examine the relationship between male mating behavior, evidence of female post-copulatory choice and variation in the male sex comb in *Drosophila bipectinata*. The first project (Chapter 1) seeks to test the hypothesis that mating male vigor (a proxy for condition) is associated with the size of the sex comb. This project was designed specifically to elucidate the mechanism(s) for differential mating success in relation to comb size in a field population (Polak et al., 2004). The possibility of female cryptic choice occurring in this system is also pursued in this chapter. Project 2 (Chapter 2) tests the hypothesis that there is a positive genetic correlation between two condition-dependent secondary sexual traits- sex combs and courtship song- which would reveal unique evidence for ‘good genes’ sexual selection. In particular, my pursuit of a genetic association between sex comb tooth number and song parameters seeks to test Rowe and Houle’s (1996) model describing how such a situation, where multiple condition-dependent traits covary positively, can exist. If the expressions of these two traits are dependent on a common pool of resources, despite their presumably different developmental pathways, then there are likely other life history traits which share links to this (for example, male mating vigor explored in Chapter 1).

Thus, by focusing on the mating dynamics of an experimentally accessible system, my hope is to contribute to the overall field of sexual selection.
REFERENCES


CHAPTER 1

In Pursuit of the Mechanisms of Male Mating Success in the Species *Drosophila bipectinata*

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ABSTRACT

Traits likely to confer mating advantages in the context of intrasexual selection include weapons for fighting, size and vigor. A correlate of male competitive ability, i.e., vigor, was hypothesized to account for the elevated mating success of males with larger combs in a previous field study. Vigor, or the capacity for exertion, was measured by the probability, latency, and duration of copulation in relation to male sex comb and body size in pairings between a single male and female. No significant relationships between these variables were detected, other than a significant effect of male body size on the probability of mating on the first of two days of experimentation. A second study sought evidence of female postcopulatory (cyptic) choice via time of oviposition and number of eggs laid in relation to sex comb and body size of the male. No significant relationships were detected.
INTRODUCTION

Non-random mating arising because of competition for mates has attracted the attention of evolutionary biologists both because of its role in sexual selection and its potential influence on genetic variation in a population (Wade and Arnold, 1980; Parker and Partridge, 1998). Non-random mating, for example, may increase the rate of evolutionary change, and even promote speciation in some circumstances (Udovic, 1980). Behavioral biologists address the causes of non-random mating because understanding the mechanisms by which variation in mating success is achieved may lead to a better understanding of the evolutionary forces underlying and/or driving sexual selection.

The primary mechanisms that generate non-random matings are male-male competition (intrasexual selection) and mate choice (intersexual selection) (Borgia, 1979; Bradbury and Gibson, 1983; Kordic-Brown and Brown, 1984; Arak, 1988; Beehler and Foster, 1988). Depending on the mechanism responsible for driving selection, certain traits will be emphasized in the process. For example, in the context of (what usually amounts to) male-male competition, traits likely to become targets of selection include weapons for fighting (i.e. antlers), large size, and vigor (Andersson, 1994). In contrast, the consequence of mate choice (with females generally considered the choosers) may be selection on traits like plumage color and pattern in males, or traits in females that bias paternity in favor of particular males, such as multiple specialized sperm storage organs, and time and rate of oviposition (Eberhard, 1996). Both theoretical and empirical studies of sexual selection have concentrated on pre-copulatory tactics when examining female choice. Female choice exerted following intromission, called post-copulatory mate
choice, can also be crucial in determining an animal’s reproductive success, as the above examples implicate, but this mechanism has been largely ignored (Eberhard, 1996).

Inter- and intrasexual selection mechanisms may work in concert with, or in opposition to natural selection, and are by no means mutually exclusive. Evidence indicates that both processes may be simultaneously important in some species (Bradbury and Davies, 1987), but quantifying the relative importance of each has proven difficult. Experimental studies are important because they can identify which male and/or female traits are used in mate discrimination, and thus which mechanisms are likely to be operating (Kirkpatrick, 1987).

The objective of the current study is two-fold: the first aim is to evaluate mechanisms of intrasexual selection for their potential to explain non-random mating in the species *Drosophila bipectinata*. Specifically, Polak et al. (2004) observed that in the field, mated male *D. bipectinata* had more teeth in the C2 region of the sex comb (independent of body size), and were larger than single (i.e., umated) males. The suggestion of a selective force acting on both sex comb and body size provided by these field observations is not surprising, given the following: 1) sex combs vary in size and position along the foretarsi both within species and between sister taxa (Nuzhdin and Reiwitch, 2000; see West-Eberhard, 1983 for a discussion on the relative pace of evolution on sexually selected traits versus non-sexually selected traits). 2) Polak & Starmer (2005) have shown that a 4˚ increase in developmental temperature increases comb fluctuating asymmetry (a purported measure of developmental instability) and reduces both sex comb tooth number and adult body size. A reduction in size or expression of a trait when a stressor (i.e., temperature change) is added to its environment
is taken as evidence of the condition-dependence of that trait. And lastly, 3) the prevalence of selection for large body size across species of all kinds (Crespi, 1989) suggests that selection may be operating on sex comb and body size. Thus, evidence for selection on males in this system is consistent with other published findings. However, the mechanisms by which differential mating success between males of varying sex comb and body size observed in the field (Polak et al., 2004) have eluded lab and fieldwork thus far, and stands as the focus of part of this project.

If intrasexual selection can explain the mating success of large (in sex comb tooth number and body size) male *D. bipectinata* relative to small (in tooth number and body) males, then vigor is predicted to be associated with these features (see below for elaboration), targeted by selection, and responsible for successful courtships. The *D. bipectinata* mating system most closely resembles a scramble competition, where there is a premium on males who are able to locate and mate with multiple females within a narrow mating period (Thornhill and Alcock, 1983). Courtship, which occurs between 0515-0700 h, entails both males and females orienting themselves so that males situate on the side or behind a female they will eventually pursue further. The male then proceeds to tap the female with his foretarsi, and move from a rear position to facing her and back again. Intermittently throughout this time, males will vibrate and scissor their wings, movements that generate stereotypic pulses of sound that females use to discern conspecifics from other species, and perhaps to gage quality of conspecifics (Crossley, 1986; Hoikkala and Isoherranen, 1997). If the female remains still at this time, the male will eventual position himself behind her, vibrate his wings and then attempt to mount her- if successful, copulation occurs.
Accordingly, the potential targets of selection during scramble competitions include rate of orientation, ability to maintain orientation on rapidly moving females, ability to distinguish virgin from non-virgin females, persistence, and vigor of courtship (Spiess, 1970; Spieth, 1974). Courtship and subsequent copulation behavior are energy-requiring processes. Thus, allocating resources efficiently in order to maximize mating effort should increase fitness in males of this system. Indeed, laboratory studies have shown positive phenotypic correlations between adult body size and fitness components (such as vigor) in Drosophila (Partridge and Fowler, 1993). A positive phenotypic correlation between male size and mating success has also been found in the field for several Drosophila species (Partridge et al., 1987a; Markow, 1988; Santos et al., 1988, 1992; Markow and Ricker, 1992). Large male body size is often associated with success during pre-copulatory competition (Otronen, 1984; Crespi, 1986, 1988; Alcock, 1996), and there is the suggestion that large males may also be better able to attract, stimulate or compel potential mates (Davidson, 1982; Hughes and Hughes, 1985; Simmons, 1986, 1988; Crean et al., 2000).

Here, prospective intrasexual influences of both male sex comb tooth number and body size are explored in a laboratory experiment designed to address these possibilities simultaneously. Males were individually paired with females, and 24 hours later those same males were paired with different females. The probability of these male flies copulating, their latency to copulate, and the duration of these matings within a pre-determined time period were all recorded. Male sex comb tooth number and body size were also recorded in order to test for a relationship between these traits and the above-mentioned response variables (i.e., probability, latency and duration of mating).
The hypothesis that pre-copulatory mating success of males with more sex comb teeth is attributable to their vigor is evaluated, then, based on the following predictions:

1) Males with more teeth in the C2 region of the sex combs, with body size controlled for, will have a higher probability of mating with virgin females in the absence of competitors than males with fewer teeth. 2) Of those that copulate, males with more teeth in the C2 region of the sex combs, independent of body size, will exhibit shorter latencies to mate with virgin females than males with fewer teeth. 3) Males with more teeth in the C2 region of the sex combs, independent of body size, will experience longer copulation durations with virgin females compared to males with fewer teeth. As body size has also been shown to influence pre-copulatory mating success in the *D. bipectinata* system (Krishna & Hedge, 1997; Polak et al., 2004), body size was expected here to significantly affect probability, latency and duration of copulation in a fashion similar to sex comb size (i.e., a bigger body should yield a higher probability of mating, shorter latency to mate, and longer duration of copulation).

The direction of these predictions are predicated on field data (Polak et al., 2004), and thus these predictions act as potential explanations for why certain males were found copulating more often than others. If males who are bigger and have more sex teeth are more likely to mate, get to a mate more quickly (shorter latency) and/or spend longer copulating with those mates, then the probability of observing them in copula (as Polak et al.’s 2004 field data found) increases.

These behavioral traits (i.e., latency and duration of copulation) are expected to have a genetic basis, and thus it is also predicted that a consistency in these traits (i.e., similar lengths of each) over time among individuals will be observed. This is despite the
fact that theoretically, mating traits should have particularly low repeatabilities due to strong environmental influences (Price and Schluter, 1991). However, the maintenance of a homogeneous environment in a laboratory setting should eliminate the influence of environmental factors, unmasking the genotypic component of the behaviors that could potentially reveal a permanent set of behaviors per individual male examined.

The expected outcomes of these tests are in large part based on literature indicating that males control latency and duration of copulation (Merrell, 1949; Kaul and Parsons, 1965; Parsons and Kaul, 1966; Macbean and Parsons, 1966, 1967; Hosgood and Parsons, 1967). Therefore, the focus is on pre-copulatory intra-sexual mechanisms as opposed to female choice models to explain the field data. This is not to suggest, however, that mechanisms of intersexual selection are not relevant or occurring in this organism, and the possibility of their operating in the D. bipectinata system has not been overlooked here.

Polak et al.’s (2004) field data similarly inspired the second aim of this study, which switches the focus from mechanisms of intrasexual selection to elucidating potential mechanisms of female choice. Instead of trying to explain why males with larger bodies and more teeth in the sex combs were observed mating more often, the consequences of pre-copulatory mating success are explored via mechanisms of female cryptic choice, or female post-copulatory influence over male reproductive success. As many females of many species mate multiply (Eberhard, 1996), copulation does not guarantee paternity of all or even any offspring. Therefore, any purported fitness advantage of males achieving pre-copulatory success is premature if associations with traits also able to confer post-copulatory success have not been identified. Mating does,
obviously, correlate with the potential for paternity, and higher mating success may increase the probability of siring more offspring. Moreover, the traits associated with mating success (i.e. size) may be heritable. Thus, bigger males with larger sex combs that were successful in accruing mates may harbor qualities that would make their sons equally successful in a mating context, and their daughters likely to prefer males with those traits. Females, then, would presumably bias their reproductive functions in order to maximize the harvest of sperm attained from attractive males, thereby indirectly increasing their fitness gains.

The hypothesis generated to explain the question of whether or not female cryptic choice may be occurring in the *D. bipectinata* system is that females manipulate their reproductive functions to bias paternity towards males with larger combs and bigger bodies. The validity of this hypothesis will be tested by exploring the latency to oviposition (time from when females complete copulation until the first egg/s are laid) and the number of eggs laid at this first bout of oviposition. No data yet are available on post-copulatory mate choice in this system. Thus, the aim of these queries is exploratory, with the experiment designed to detect a potential signature of post-copulatory sexual selection. The identification of any patterns, for example, if females mated to males with more sex teeth laid eggs sooner, could propel future studies regarding proximate explanations of such phenomena, i.e. mechanisms of sperm storage and female remating rate in this species.

**MATERIALS AND METHODS**

**Stock and rearing conditions** *Drosophila bipectinata* stocks were initiated in 2003, where they were field-caught in Cape Tribulation, northeastern Australia, and
then maintained in the laboratory at the University of Cincinnati in half-pint bottles at room temperature. The bottles contained 12 grams of instant *Drosophila* medium (Carolina Biological Supply Co.), 53 ml distilled water, 4-5 mg of Fleischmann’s active dry yeast and 0.5 grams of banana. To collect virgin males and females for each experiment, the bottles were cleared of animals using a light dose of carbon dioxide anesthesia after adults were added just three days prior. This ensured that both the age of the emerging pupae was relatively similar, and that moderate larval densities were maintained. Flies were collected via aspirator no more than 3 hours after eclosion to ensure virginity. The sexes were separated and placed in 35ml vials containing yeast, 1.7 grams instant *Drosophila* medium, 8 ml water and banana slurry. No more than 10 flies were kept per vial. Flies were housed in incubators set at a 12 (25ºC): 12 (23ºC) L:D photoperiod and aged for 72 hours.

**Hypothesis 1: Pre-copulatory mating success is attributable to male vigor**

*Measuring probability, latency and duration of mating* Three day-old virgin males were dispersed with an aspirator into individual agar vials that were positioned side-by-side across the length of a table. Only one male was added to each vial in order to eliminate the possibility of male-male interactions (physical and/or chemical). After a brief acclimation period, 3 d old single, sexually mature virgin females were introduced into the experimental vials via aspirator. Virgin females were used because they were expected to be much more motivated to mate than inseminated females, and so less likely to exert choice on their first mate partners (Andersson, 1994). This helps to alleviate some of the potential for confounding inter- and intra-sexual models of selection. The
strength of male vigor was inferred behaviorally by recording 1) the occurrence of mating, 2) copulation latency (time passed from introduction of female to start of copulation) and 3) copulation duration (time passed from start to end of copulation). After copulation or an hour had passed without copulation, the trial was terminated for that male, who was then transferred to a fresh vial. In order to estimate repeatability, the same males paired initially were kept overnight and paired with different 3 d old virgins from the same stock, 24 h after the males’ first pairing. The same measurements (latency and duration) were taken. The two-day period over which males were assayed corresponds to 1 block of experimentation. This study culminated in running 6 blocks with a total of 348 males tested. All experimental subjects (male and female) were sacrificed and placed under an Olympus SZX12 stereomicroscope to obtain thorax length (distance from the anterior edge of the thorax to the distal end of the scutellum), a reliable estimate of body size (Lefranc and Bundgaard, 2000). These measurements were taken using an ocular micrometer. Sex-comb tooth number measurements were taken by removing the foretarsi of each male and placing them on a microscope slide that was positioned atop a white background, which enabled a more prominent view of the combs.

**Data Analysis**

A preliminary ANOVA was conducted in order to test the effects of block (1-6) on log-transformed latency to copulate (time from when males are entered into the vial to when copulation begins) and duration of copulation (time from beginning of copulation until the pair disengage). For the males that copulated twice, mean times of latency and of duration of copulation in both mating events were used in the analysis. Because block
had a significant effect on both latency to copulate and duration of copulation (see Results), residuals from analyses of block effects on these variables were used in all regression analyses, and block was added as a factor in subsequent ANCOVAs.

Female thorax lengths were initially added as a covariate in all models during the data exploration phase of this study. This variable did not significantly impact latency or duration of copulation, and thus was taken out of final analyses.

Separate, preliminary ANCOVAs were conducted in order to test the assumption of homogeneity of slopes. Significant interactions between male thorax length (covariate), C2 (number of teeth in this region of the sex comb only), and the copulation categories (copulated not, and copulated either 1 or 2 times) were tested on the dependent variables of residual latency to copulate and residual duration of copulation. There were no significant effects detected, which verified the homogeneity of slopes assumption. Other non-significant findings in this model were not included in the final analysis.

Variance components derived from a one-way ANOVA were used to calculate the repeatability of latency and duration of copulation after the procedure described by Lessells and Boag (1987).

*Prediction 1: Males with more teeth in the C2 region of the sex comb and larger bodies will have a higher probability of mating* In order to evaluate this prediction, a multiple logistic regression was performed where a dichotomous response variable consisting of males who did and did not copulate was regressed on the independent variable C2 with covariates male and female thorax lengths. This analysis evaluated male performance on both days of the experiment. The expectation was that males with larger
C2s and larger bodies would be more frequent in the ‘copulated’ category than smaller males with fewer C2 teeth.

A similar test of the prediction that larger males (in comb and body) would have a higher probability of mating was conducted with a more refined model, where males were partitioned into three categories: mated once, twice or not at all. With block as a factor and male thorax length as a covariate, these groups were tested in an ANCOVA to see whether they were significantly different in terms of C2 and thorax length. The expectation was that males that copulated most often would be larger in comb and body.

*Prediction 2: Males with more teeth in the C2 region of the sex comb and larger bodies will demonstrate a shorter latency to mate*  In order to test this prediction, two regressions were performed, the first of which explored the potential predictive power of C2, where residual latencies to copulate were regressed onto C2 values. The residuals were derived from a one-way ANOVA of block on latency to copulate. The use of these residuals ensured that any variation in latency between blocks was controlled for in analyses. The second regression focused on male thorax length as the independent variable, with residual latency to copulate as the response variable. It was predicted that C2 and male thorax length would have a significant inverse relationship with latency to copulate such that the larger the independent variables (C2, thorax length), the shorter the latency to copulate. For the purposes of generating figures, the mean latency value from all males assayed was added to residual latency measurements to aid interpretability of the graphed results.
Another test of the prediction that C2 and male thorax length affect latency to copulate involved the use of general linear models. An ANCOVA was performed in which males possessing total teeth numbers in the C2 region above the average of the males assayed were characterized as ‘high,’ and males having total C2 teeth numbers below the average were characterized as ‘low.’ These C2 categories were entered into the model with block and block X C2 category interaction term entered as factors. This model aimed to test for significant differences between the ‘high’ and ‘low’ male C2 categories with respect to their latencies to copulation (response variable).

A similar model was also constructed with the exception that the categorical variable was instead comprised of males characterized as ‘high’ or ‘low’ based on their thorax length measurements (above the average length = ‘high’, below average= ‘low’). Other independent variables in this model included block and a block X thorax length interaction on the dependent variable, latency to mate. ‘High’ males were expected to have a significantly shorter latency to copulate than ‘low’ males.

**Prediction 3:** Males with more teeth in the C2 region of the sex comb and larger bodies will demonstrate a longer duration of copulation

Two regressions were generated in order to test the prediction that male sex comb and body size positively affects duration of copulation. In both analyses, the response variable consisted of the residuals from the significant effect of block on copulation duration. One analysis regressed residual copulation duration on C2. The other analysis regressed residual copulation duration on thorax length. Mean duration of copulation was added to all residual duration measurements for reasons described previously. The results of these analyses were
predicted to reveal a significant correlation between C2, male thorax length and duration such that larger C2 and male thorax length measurements would be responsible for longer durations of copulation.

An ANCOVA model was constructed with the categorical variable consisting of ‘high’ and ‘low’ sex comb-sized males, as described above. The model also contained block and a block X C2 interaction term. This analysis explored whether the ‘high’ and ‘low’ categories of males could be distinguished based on their durations of copulation, with the prediction that ‘high’ males would have significantly higher average copulation durations than ‘low’ males.

A second ANCOVA sought effects of male thorax length on the duration of copulation. ‘High’ and ‘low’ categories were as described previously (i.e., above average = ‘high’, below average = ‘low’). In addition, both block and a block X thorax length interaction term were included as independent variables. It was expected that the ‘high’ and ‘low’ category males would differ significantly in terms of this particular behavior (duration).

**Hypothesis 2: Females manipulate reproductive functions according to male phenotype**

*Female temporal fertility* To test for evidence of female post-copulatory behavior, whereby females manipulate the sperm they receive in order to bias the paternities of their offspring, mated females (from Hypothesis 1) were observed with regard to their reproductive output. After each day of experimentation, the females who mated were swiftly removed from the experimental vials and transferred to new vials. These vials
were checked 3 times a day (including the time immediately after the experiment) and the following data were recorded: 1) presence of eggs, 2) time of oviposition (end of copulation to time deposited egg/s were first observed), and 3) amount of eggs laid at first oviposition event. These procedures were carried out for the first 4 blocks of Hypothesis 1. Females who had not yet oviposited were transferred to fresh vials every three days until oviposition occurred, or they died. If mold had developed in their vials (a rare occurrence), females were transferred into new vials earlier than three days.

Data analysis

A preliminary ANOVA was conducted to test for the effects of block (1-4) on the dependent variables: 1) latency to oviposit (time from immediately after copulation until eggs are first observed); 2) and mean number of eggs laid at the time of that first oviposition event. For males who copulated on both days, average latency to oviposit and average number of eggs laid were calculated for both of his partners. Because there were significant effects of block on latency to oviposit and eggs laid at time of the first oviposition event (see Results), the residuals from these analyses were used in regressions, and block was added as a factor in subsequent general linear models.

Exploring the interaction between the covariates male thorax length and female thorax length with block on the dependent variables latency to oviposit, and number of eggs laid at that time verified the homogeneity of slopes assumption. This negated any need to pursue these variables further in ensuing analyses.
Prediction 1: Latency to oviposit will be influenced by male sex comb tooth number and body size.

Two regressions were conducted in order to test the predictive power of both male tooth number and body size on latency to oviposition. The first analysis regressed residual latency to oviposit on C2. The second analysis regressed residual latency to oviposit on male thorax length. In order to plot positive values, mean latency was added to all residual measurements. A significant relationship between C2, male thorax length and time to oviposition was predicted.

Two ANCOVAs were performed, the first to test the prediction that sex comb tooth number affects latency to oviposit, and the second to test for male size effects on the same response variable. For the first analysis, males were characterized as either ‘high’ or ‘low’ according to C2 tooth count. The ‘high’ and ‘low’-category males, block, and a block X C2 interaction term are independent variables while the response variable was latency to oviposit. The second analysis testing effects of male body size on oviposition latency had males characterized by thorax length into the same ‘high’ and ‘low’ categories described previously. Block, and a block X male thorax length interaction were similarly included in this model.

It was expected that the male category (‘high’ and ‘low’) with respect to both C2 tooth measurements and thorax lengths would significantly affect latency to oviposit, lending credence to the hypothesis that females modify their reproductive efforts based on male sex comb tooth number and body size.

Prediction 2: Mean number of eggs laid at the first oviposition event will be influenced by male sex comb tooth number and body size.

The residuals from the effect of
block on mean eggs laid at first oviposition event were regressed onto C2, and in a separate analysis regressed onto male thorax length. C2 and male thorax length were predicted to significantly affect mean number of eggs laid. The scatter plots of these analyses were designed as above, with the addition of mean number of eggs laid on the resulting residual measurements.

An ANCOVA was performed in which the categorical variable was ‘high’ or ‘low’ sex comb (C2) size, as described above. These categories, along with a female thorax length covariate (male thorax length was not included in the final model after preliminary tests showed it had no significant effects), block (factor), and a block X C2 interaction term were tested on the dependent variable, mean number of eggs laid at the first oviposition event. A second ANCOVA with males characterized as ‘high’ and ‘low’ based on thorax length measurements was also conducted.

Significant differences in the number of eggs laid between these groups would suggest that females may be employing a post-copulatory strategy in this system, influenced either by variation in male tooth number and/or male body size.

All statistical analyses (from Hypotheses 1, 2) were computed with SAS statistical programming (2002).

RESULTS

Hypothesis 1: Pre-copulatory mating success is attributable to male vigor

In the experiment, 331 out of 348 males (95%) copulated on either 1 or both days during this experiment; 200 of those males mated on both days.

Two ANOVAs, which tested the effect of block on latency to copulate and duration of copulation, were significant (latency: $F_{5,333} = 5.83, p < .0001$; duration: $F_{5,331}$
\[ F = 10.27, p < .0001 \]. In order to control for this variation between blocks, regression analyses with latency and duration as response variables were replaced with residuals from the ANOVAs described above (see Data analysis section).

**Prediction 1:** Males with more C2 teeth in the sex combs and who have larger bodies will have a higher probability of mating

I tested to see whether male groups could be differentiated on the basis of probability of mating on either the first or second day of experimentation. The multiple logistic regression showed that C2 did not have any significant predictive value on either day (Table 1). Male thorax length, however, did significantly correlate with these categories, with larger males copulating less often than smaller males on the first day of experimentation, but size had no effect on the second day (Table 1). Therefore, these analyses do not support that C2 would affect probability of mating, and that larger males would be more likely to mate than smaller males.

An ANCOVA with males categorized by mating activity into three groups, copulated once, two times or not at all, showed no significant differences in C2, or thorax length between them (Table 2; Fig.1). Thus, the prediction that larger males with relatively more C2 teeth would have a higher probability of mating was not supported here.

**Prediction 2:** Males with more C2 teeth in the sex combs and with larger bodies will demonstrate a shorter latency to mate

According to the regression analyses, neither male C2 counts nor thorax length could significantly predict latency to copulation for the males studied (Table 3; Fig. 2). Males were then characterized as ‘high’ or ‘low’
according to their total C2 teeth values in one analysis, and by their thorax length measurements in another. These groups were tested to see whether they could be differentiated on the basis of their latencies to copulate. There were no significant differences between the ‘high’ and ‘low’ males in the amount of time it took them to copulate in either analysis (Table 4; Fig. 3). The prediction that C2 or thorax length would affect latencies to copulate was not supported by the data.

**Prediction 3: Males with more C2 teeth in their sex combs and who have larger bodies will demonstrate a longer duration of copulation**

Residual duration of copulation was regressed onto C2, and in a separate analysis, on male thorax length. There was no relationship between C2, male thorax length and duration of copulation, contrary to what was predicted of these variables (Table 3; Fig. 4). Males were also characterized as ‘high’ or ‘low’ according to their total C2 tooth numbers, and then by thorax length measurements, and tested to see whether these groups differed on the basis of their durations of copulations. There were no significant differences in durations of copulations between ‘high’ and ‘low’ males, whether sorted by C2 or thorax length (Table 4, Fig. 5). Thus, the prediction regarding these groups was not supported.

The repeatabilities that were estimated by calculating the intra-class correlation obtained from a one-way ANOVA were not significant for latency (R= -0.006, $F_{1,455} = 0.46, \text{NS}$) or duration of copulation (R= -0.007, $F_{1,454} = 0.39, \text{NS}$). Therefore, the prediction regarding the repeatability of behavior among males over time was not supported.
Hypothesis 2: Females manipulate reproductive functions according to male phenotype

Of a total of 227 mated females, 209 (92%) laid at least 1 egg. The results of two ANOVAs exploring effects of block on latency to oviposit, and mean number of eggs laid at that point were significant (latency to oviposit: $F_{3,209} = 11.15, p <.0001$; mean eggs laid: $F_{3,209} = 14.29, p <.0001$). Therefore, the residuals from these analyses were used in regressions, and block was added as a factor in subsequent analyses of variance.

Prediction 1: Latency to oviposit will be influenced by male sex comb and body size

Two regressions were conducted, one with C2 as an independent variable, the other with male thorax length as an independent variable, and both with residual latency to oviposit as the response variable. In both analyses, non-significant effects of the independent variables were found (Table 5; Fig.6)

Two ANCOVAs with ‘high’ and ‘low’ category males, based first on C2, then on thorax length, tested whether these variables could explain variation in female latency to oviposit. No significant effects were discovered (Table 6; Fig. 7). There was no support, then, for the prediction that significant differences between latencies to oviposit and either male sex comb tooth number or body size exist.

Prediction 2: Mean number of eggs laid at the first oviposition event will be influenced by male sex comb tooth number and body size

The prediction that the number of eggs laid at the first oviposition event would be associated with C2 numbers and male thorax length was not supported, as demonstrated in two regression analyses (Table 6;
Fig. 8). Males assembled into groups based on both total C2 tooth numbers (either ‘high’ or ‘low’) and thorax length measurements (also ‘high’ and ‘low’, but in a separate analysis) also did not influence the mean number of eggs laid at that first oviposition event (Table 6; Fig. 9). These data do not support the prediction that sex comb tooth number or thorax length significantly affects the number of eggs oviposited. Female thorax length, however, did significantly affect the number of eggs oviposited with larger females laying more eggs (Table 6).

DISCUSSION

**Hypothesis 1: Pre-copulatory success is attributable to male vigor**

That males with larger teeth numbers were found mating more often than those with fewer teeth in the field was not explained here by either their latency to, or duration of copulation. Males with more teeth in this study were not even more likely to mate than those with relatively fewer sex teeth.

The first aim of this study attempted to isolate the mechanism responsible for the apparent non-random mating observed in a field study of the species *D. bipectinata*. Polak et al. (2004) found male *D. bipectinata* with more teeth in the C2 region of their sex combs mating more often than males with fewer teeth in that same region. I hypothesized that these differences in pre-copulatory success would be attributed to differences in male vigor, such that males with more sex comb teeth exert more effort (delegate more resources towards mating) than males with fewer teeth. The addition of a laboratory component in an attempt to explain these findings, with male vigor reflected in latency to and duration of copulation, proved ineffective.
Male thorax length was also shown to associate with a higher mating probability in Polak et al.’s (2004) field study, independent of comb size. Therefore, body size was predicted to have effects on latency and duration of copulation similar to those predicted for comb size: larger males should exhibit shorter latencies to mate, and longer durations of copulation. But like comb size, male body size did not reveal any such effects. Larger males, however, were less likely to mate on the first, but not the second, day of experimentation.

It is curious that the effect of size varied from the first to the second day. On both days, males were paired with 3 d old virgin females in exactly the same type of vial at the same time of day. It was this consistency in experimental design that led to the expectation that the behavior of a male on the first day would be repeated on the next day. However, quantifying this prediction via repeatability statistics showed non-significant findings: variation in both latency and duration of copulation among individuals was greater than the between-individual variation. There were, however, slight differences in the circumstances surrounding the pairings on both days which may account for lack of repeatability. For example, prior to their first pairing, males were kept in vials of about 10 individuals. After that first pairing, males were kept singly until they were paired again 24 hours later. Males were housed individually after that first pairing in order to track them, but the isolation might have affected their performance on the next day. It has been shown that males display more courtship towards virgin females after a period of isolation than males who were not socially isolated (Kim and Ehrman, 1997). Also, it may be that 4 d old males are more sexually mature, and thus more inclined to attempt mating. In addition, males on the second day had at this point been
previously exposed to females, whereas they had not been prior to their first pairing. There is the suggestion, then, that there may be a learning component, wherein performance is enhanced with experience, something commonly found in *Drosophila* species (Siegel & Hall, 1979; Gailey et al., 1982, 1985; Tompkins et al., 1983; Dukas, 1999, 2004, 2005). These suspicions find support in the data that generally show more males mating on the second day of experimentation than on the first: in 4 of the 6 blocks conducted, males were anywhere from 9 to 53% more likely to mate on the second day than on the first day of experimentation.

The significant inverse relationship between size and mating success that surfaced on the first day of experimentation, which more closely simulated a natural situation anyway (males were not completely isolated as they would not be in the wild), is intriguing nonetheless. Although, contrary to what was expected based on Polak et al. (2004), in species who engage in scramble competition (Thornhill and Alcock, 1983) there is both theoretical and empirical support for higher mating success of small males over larger ones. Given that in a scrambling species rapid location of a mate is crucial, Andersson (1994) hypothesized that characters linked with searching and mobility would be important, and that sexual selection would favor small body size and agility. Several studies support this hypothesis (e.g. Banks and Thompson, 1985; McLachlan and Allen, 1987; Neems et al., 1990; Blanckenhorn et al., 1995; Dunn et al., 1999; Moya-Laraño et al., 2004). In contrast, there is ample evidence in the literature demonstrating the success of large flies in a mating context (Thornhill and Alcock, 1983). This has been attributed to intra-sexual selection in the form of aggression or scramble competition for receptive females, or because, being faster and more active, bigger males not only encounter more
receptive females than do small males, but are also better able to follow females when they move during courtship (Partridge et al., 1987a).

The divergent evidence concerning the consequences of size is not confined to studies between species; studies within and among populations of the same species produce conflicting results regarding the importance of the size of the male in mating success (for instance Partridge et al., 1987a and Markow and Ricker, 1992 for *D. pseudoobscura*; Partridge et al., 1987a, Markow, 1988 and Joshi et al., 1999 for *D. melanogaster*). Also, as more species are studied, the number of examples where body size and aspects of male mating success (i.e. latency and duration) are uncorrelated is increasing (Markow et al., 1996).

One potential explanation for discrepancies or differences in latency among studies have been found elsewhere is that these results are a consequence of how latency is defined. Brown (1964), Spiess (1968), Spieth and Ringo (1983) and others have defined latency to copulate as the time from the beginning of courtship until copulation occurs. Latency to copulate was defined in this study as the time from first exposure of the male to the female until copulation occurs. This distinction could become important in instances where copulation occurs with little courtship. For example, there were situations observed during this study where males remained relatively stationary when paired, while their cohorts were actively courting in adjacent vials, for minutes at a time. In extreme cases, those quiescent males would then lunge onto the back of the female (less extreme situations involved a few wing flutters, or some taps with the foretarsi and then a successful mounting attempt) and then, with seemingly little pre-amble, were immediately engaged in copula. At the same time, some males who did court vigorously
were not even guaranteed a mating for their efforts. So, while two individuals may have similar latency measurements, those times may not accurately reflect the effort put forth by each male. In addition, effort may not correlate as strongly with mating success as presumed, but instead be influenced by the particular social circumstance of that mating. We only considered intra-sexual mechanisms for this part of the study- if females evaluate the quality of male signals (i.e. show mating preferences) which we did not consider here, then they should take longer to accept males that lack certain mating criteria and mate sooner with males having ‘better’ signals (Droney, 1996). Perhaps latency to copulate is not as completely male-mediated as has been suggested (Merrell, 1949; Kaul and Parsons, 1965; Parsons and Kaul, 1966; Macbean and Parsons, 1966, 1967; Hosgood and Parsons, 1967), but in part is a reflection of the receptivity of the female being courted. A closer analysis of the behavior of individual flies courting under a similar set-up, for example with video-recordings, would have provided a way to quantify these predictions.

As mentioned previously, the success of large males is sometimes the effect of intra-sexual competition. If larger males can out-maneuver smaller ones, as has been found in studies of Drosophila species, (Krebs and Barker, 1991; Norry et al., 1995), then, in the absence of any competition, smaller males may be expected to perform just as well in certain aspects of mating as larger ones. Males were segregated in this study in order to assess intrinsic latency and duration measurements without the addition of other males to affect these variables. However, eliminating male-male interactions from the study might have taken out the very element that elicits differential responses. Perhaps more energy is expended on intra-sexual conflict than on the courting process. Another
possibility is that large comb and body size act as signals to other males, communicating their prowess so that relatively smaller males opt to “pick fights” they are more likely to win (i.e. with males their own size).

Like other components of mating, duration of copulation and the direction (longer or shorter) it takes with regard to size has produced mixed results. In this particular case, the direction of the prediction was generated based on the 2004 field study of the species. While field findings of Polak et al. (2004) showed that males who had more teeth in the C2 region and larger bodies were copulating more often, they acknowledge that the experimental design did not take into account the fact that males considered ‘single’ (unmated- see Polak et al., 2004) might have previously mated, or that their capture interrupted subsequent matings. Therefore, the prediction that males with larger sex combs and larger bodies would have a longer duration of copulation in this study is premised on the idea that, while big and small males mate in the field, bigger males were “caught” more often because they averaged longer copulation durations, increasing the opportunity for detection. The current project did not find variation in copulation duration among males as expected, but this may be a reflection of the experimental design. Work on the water strider, Gerris buenoi (Rowe and Arnqvist, 1996; Arnqvist and Danielsson, 1999; Danielsson, 2001), as well as on the dung flies, Scathophaga stercoraria (Ward and Simmons, 1991; Parker and Simmons, 1994, 2000; Parker et al., 1999) has shown that copulation duration tends to vary with male size and their perception of mating opportunities. Our experimental design entailed exposing all males to one female a day, so that, regardless of phenotype, they all had the same number of potential mates. And this way, males might have been less inclined to modify their behavior with any one
female according to any other perceived mating opportunities. If males were continuously exposed to females, or exposed to multiple females at a time thereby emulating a more realistic scenario, then differences in duration may have become apparent.

Rearing conditions may also explain the lack of finding differences in latency and duration of copulation between large (in comb and body) and small males. Flies in this study were cultured under low density conditions to reduce the environmental component of variation in sex combs, body size and mating activity. As pointed out by Joshi et al. (1999), the range of size variation in populations kept at low density is small compared to that in laboratory populations cultured without controlling larval densities. These authors stressed the importance of density of culturing to various fitness components in *Drosophila* and stated that differences in results of *Drosophila* studies can often be due to inadvertent differences in culture densities. The laboratory environment is generally designed to produce healthy flies and to maximize reproductive output (Gromko and Markow, 1993). Thus, for any correlation observed between sex combs, body size and mating activity, the expectation was that it was due mainly to a genetic component. Under the laboratory conditions of this study, the levels of mating activity (i.e. latency and duration) did not appear to be influenced by comb or body size in *D. bipectinata*. In nature, on the other hand, feeding and breeding sites may not always be abundant. This implies that environmental conditions found by the flies are extremely variable and suboptimal, and the reproductive potential of the flies in these environments is inferior to that observed in laboratories (Bouletreau, 1978). In the Hawaiian *Drosophila, D. grimshawi*, males fed high-protein diets were in better physical condition, courted more
vigorously, and mated sooner and more often than males fed low-protein diets (Droney, 1996). In *D. buzzatii* male body size (as measured by wing length) had no impact on mating success in competition among males that grew up under un-crowded conditions, but size was important among males that grew up under crowded conditions (Santos, 1996).

This particular study is in agreement with the suggestion of Joshi et al. (1999) that it is likely that environmentally-induced variance in body size may be important in determining the mating success of *Drosophila* species in the wild. It is thus possible that the mating success of a wild male, which is presumably a culmination of the time it spends interacting with other males and in pursuing females (Partridge et al., 1987a, b), will be influenced by external conditions such as the quantity and quality of food consumed by the individual at the larval stage, which will affect its final size (Santos, 1996). This environmental effect in some field studies could have produced an inconsistent relationship between male size and mating success (see Markow et al., 1996). The ability of males to perform vigorous courtship displays under stringent environmental conditions, for example, might be a key indicator of male mating quality to females (e.g. Knapp and Kovach, 1991). Thus, realistic laboratory studies of sexual selection should include analyses of mating signals in the presence of realistic environmental variation (Droney, 1996).

Our results suggest that, for *D. bipectinata*, sex comb tooth number and mating activity are uncorrelated, which means that selection based on one trait will probably not affect the other. Thus, the findings of a higher mating probability associated with larger comb and body size (Polak et al., 2004) may be the result of different selection pressures.
While not statistically significant, an inverse pattern that emerged from the present study between high and low category males for C2 and thorax length, in relation to both latency (Fig. 3) and duration (Fig. 5), supports the idea that each trait may be selected for in different contexts. The present results with regard to body size are consistent with the observations of Markow et al. (1996), who found that the relationship between body size and male success is complex and may not be as strong as previously believed (see Partridge et al., 1987a, b; Wilkinson, 1987; Markow, 1988; Santos et al., 1988; Markow and Ricker, 1992; Markow and Sawka, 1992; Hedge and Krishna, 1999), suggesting that directional sexual selection for large male body size is not universal in *Drosophila*.

**Hypothesis 2: Females manipulate reproductive functions according to male phenotype**

The proposed relationship between male phenotype and female reproductive functions was not supported by the laboratory data obtained here—neither male sex comb tooth number nor body size significantly influenced the latency of the females they mated with to oviposit or the number of eggs laid at that first egg-laying event.

The second objective of this study explored the possibility of a link between the pre-copulatory success of large males with large C2 regions in the field and potential female post-copulatory strategies. Specifically, a relationship between male comb/body size and female latency to oviposit and the number of eggs laid at that time was investigated. Any response detected in these behaviors was assumed to be attributable to female control.
While this study was an attempt to uncover evidence of female cryptic choice, the assay chosen to detect it was not, in retrospect, ideal. Female cryptic choice is premised on the idea that the female has multiple mates, and preferences, and thus can manipulate the ejaculate that she receives in a manner that would be most conducive to her achieving the highest possible fitness gain (Eberhard, 1996; Ben-Ari, 2000; Birkhead and Pizzari, 2002). This study may not have provided the appropriate criteria on which to assess this question. As an example, females here were aged for 3 days before being introduced to a male. It is unlikely that a female remains virgin for that long in the field— in fact, D. melanogaster females are said to reach sexual maturity only when they are 24- to 40-hours old (Manning, 1967). During that period, however, the immature, unreceptive females are vigorously courted by males (Manning, 1967; Dukas, 2005). Furthermore, although recently mated female D. melanogaster typically become unreceptive and less attractive to males for several days, the females are still courted frequently (Manning, 1967; Cook and Cook, 1975; Dukas, 2005). So, females typically experience courtship by numerous males before they reach sexual maturity, they then may encounter several males before they choose to mate, and long-lived females may gain considerable experience, which could be employed in their subsequent mate choices. This exposure to males offers females the opportunity to learn, and females may rely on this information when choosing their mates. The first exposure to males females had here was when they were first paired with one. Therefore, females may not have discriminated against males here because they lacked prior experience with them.

Since females were paired one time, they were not given the opportunity to choose a mate, nor were they offered the possibility to remate. In such circumstances,
there is no opportunity to exert choice between ejaculates, and little choice as to how best to utilize the only ejaculate received- use it, i.e. fertilize as many eggs as possible with it. Therefore, it may be unreasonable to expect differences among females mated one time in a no-choice situation in terms of their reproductive functioning. And, if differences were apparent, those might be indicative of male-male strategies (i.e. contents of ejaculate) as opposed to female cryptic choice, per se. A better test of the possibility of female cryptic choice occurring in this system would be to present females with subsequent mating opportunities, and with the use of genetic markers look for differences in rates of paternity of the different males.

It was presumed that oviposition rate and the number of resulting eggs were correlates of fitness, justifying why females would bother adjusting these variables. However, these are just a few possible links- egg size, whether eggs were even fertilized, viability, and attractiveness of the offspring are among other traits that have fitness consequences, but were not taken into consideration here. Therefore, cryptic strategies may be employed by female *D. bipectinata* in ways other than by what was measured in this study. This leaves several other possible avenues to exhaust before concluding anything about the existence of post-copulatory cryptic choice among females in this system.
REFERENCES


Simmons LW, 1986. Female choice in the field cricket *Gryllus bimaculatus*. Anim Behav 34:1463-1470.


Table 1. Results of a multiple logistic regression, which explored whether teeth in the C2 region of the combs, or male body size could significantly predict the occurrence of copulation on either of two days of experimentation.

<table>
<thead>
<tr>
<th>Day</th>
<th>Source</th>
<th>Response trait</th>
<th>df</th>
<th>Wald statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Block</td>
<td>Cop y/n</td>
<td>5</td>
<td>31.906</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td></td>
<td>1</td>
<td>1.604</td>
<td>0.205</td>
</tr>
<tr>
<td></td>
<td>Male thorax</td>
<td></td>
<td>1</td>
<td>3.910</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>Female thorax</td>
<td></td>
<td>1</td>
<td>0.0082</td>
<td>0.928</td>
</tr>
<tr>
<td>2</td>
<td>Block</td>
<td>Cop y/n</td>
<td>5</td>
<td>9.886</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td></td>
<td>1</td>
<td>1.938</td>
<td>0.164</td>
</tr>
<tr>
<td></td>
<td>Male thorax</td>
<td></td>
<td>1</td>
<td>0.414</td>
<td>0.520</td>
</tr>
<tr>
<td></td>
<td>Female thorax</td>
<td></td>
<td>1</td>
<td>0.376</td>
<td>0.540</td>
</tr>
</tbody>
</table>
Table 2. Two ANCOVAs exploring the relationship between males copulating 0, 1 or 2 times and either their C2 teeth counts, or thorax length measurements.

<table>
<thead>
<tr>
<th>Source</th>
<th>Trait</th>
<th>r²</th>
<th>df</th>
<th>ms</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>C2</td>
<td>0.303</td>
<td>5</td>
<td>37.401</td>
<td>18.21</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Cop (0, 1, 2)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>Male thorax</td>
<td>0.656</td>
<td>5</td>
<td>0.145</td>
<td>109.43</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Cop (0, 1, 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td></td>
<td>0.0018</td>
<td>1</td>
<td>1.38</td>
<td>0.242</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>0.0013</td>
<td>341</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Regression analyses of the effects of teeth in the C2 region of the combs and male thorax length measurements on both log-transformed latency and duration of copulation.

<table>
<thead>
<tr>
<th>Source</th>
<th>Trait</th>
<th>$r^2$</th>
<th>$b \pm SE$</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td>Latency</td>
<td>0.004</td>
<td>0.035 ± 0.031</td>
<td>1.15</td>
<td>0.2499</td>
</tr>
<tr>
<td>Male thorax</td>
<td></td>
<td>0.003</td>
<td>-1.011 ± 0.956</td>
<td>-1.06</td>
<td>0.291</td>
</tr>
<tr>
<td>C2</td>
<td>Duration</td>
<td>0.0002</td>
<td>-0.003 ± 0.012</td>
<td>-0.24</td>
<td>0.8107</td>
</tr>
<tr>
<td>Male thorax</td>
<td></td>
<td>0.001</td>
<td>0.185 ± 0.366</td>
<td>0.5</td>
<td>0.6147</td>
</tr>
</tbody>
</table>
Table 4. Results from several ANCOVAs where males characterized as ‘high’ and ‘low’ in terms of C2 tooth numbers and male thorax lengths were tested to see if they produced significantly different latencies and durations of copulation.

<table>
<thead>
<tr>
<th>Source</th>
<th>Trait</th>
<th>$r^2$</th>
<th>$df$</th>
<th>$ms$</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>Latency</td>
<td>0.094</td>
<td>5</td>
<td>0.831</td>
<td>0.76</td>
<td>0.577</td>
</tr>
<tr>
<td>Block*C2</td>
<td></td>
<td>0.047</td>
<td>6</td>
<td>0.847</td>
<td>0.78</td>
<td>0.588</td>
</tr>
<tr>
<td>High/Low(C2)</td>
<td></td>
<td>1.569</td>
<td>1</td>
<td>1.44</td>
<td>0.231</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>292</td>
<td></td>
<td>5087</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>Latency</td>
<td>0.129</td>
<td>5</td>
<td>1.006</td>
<td>0.94</td>
<td>0.458</td>
</tr>
<tr>
<td>Block*thorax</td>
<td></td>
<td>1.574</td>
<td>6</td>
<td>1.47</td>
<td>0.28</td>
<td>0.308</td>
</tr>
<tr>
<td>High/Low(thorax)</td>
<td></td>
<td>1.119</td>
<td>1</td>
<td>1.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>293</td>
<td></td>
<td>1.074</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>Duration</td>
<td>0.281</td>
<td>5</td>
<td>0.228</td>
<td>2.03</td>
<td>0.074</td>
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<td>Block*C2</td>
<td></td>
<td>0.134</td>
<td>6</td>
<td>1.19</td>
<td>0.311</td>
<td></td>
</tr>
<tr>
<td>High/Low(C2)</td>
<td></td>
<td>0.044</td>
<td>1</td>
<td>0.39</td>
<td>0.534</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>294</td>
<td></td>
<td>82951</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>Duration</td>
<td>0.199</td>
<td>5</td>
<td>0.075</td>
<td>0.67</td>
<td>0.648</td>
</tr>
<tr>
<td>Block*thorax</td>
<td></td>
<td>0.060</td>
<td>6</td>
<td>0.53</td>
<td>0.783</td>
<td></td>
</tr>
<tr>
<td>High/Low(thorax)</td>
<td></td>
<td>0.188</td>
<td>1</td>
<td>1.66</td>
<td>0.198</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>295</td>
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<td>0.113</td>
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</tr>
</tbody>
</table>
Table 5. Results from regression analyses exploring the relationship between teeth in the C2 region of the combs and then male thorax lengths on both latency to oviposition, and the mean number of eggs laid at that event.

<table>
<thead>
<tr>
<th>Source</th>
<th>Trait</th>
<th>$r^2$</th>
<th>$b \pm SE$</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td>Latency to oviposition</td>
<td>0.002</td>
<td>-0.827 ± 1.171</td>
<td>-0.71</td>
<td>0.48</td>
</tr>
<tr>
<td>Male thorax</td>
<td></td>
<td>1.171</td>
<td>13.977 ± 42.017</td>
<td>0.33</td>
<td>0.74</td>
</tr>
<tr>
<td>C2</td>
<td>Mean egg number</td>
<td>0.000002</td>
<td>-0.004 ± 0.199</td>
<td>-0.02</td>
<td>0.98</td>
</tr>
<tr>
<td>Male thorax</td>
<td></td>
<td>0.0002</td>
<td>1.417 ± 7.165</td>
<td>0.20</td>
<td>0.84</td>
</tr>
</tbody>
</table>
Table 6. The effect of male categories (‘high’ and ‘low’ in terms of C2 tooth number and thorax lengths), on latency to oviposit and mean number of eggs laid.

<table>
<thead>
<tr>
<th>Source</th>
<th>Trait</th>
<th>$r^2$</th>
<th>$df$</th>
<th>$ms$</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latency to oviposition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td></td>
<td>0.168</td>
<td>3</td>
<td>2052.86</td>
<td>2.72</td>
<td>0.046</td>
</tr>
<tr>
<td>Block*C2</td>
<td></td>
<td>0.142</td>
<td>4</td>
<td>1065.55</td>
<td>1.41</td>
<td>0.231</td>
</tr>
<tr>
<td>High/Low(C2)</td>
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<td></td>
<td>1</td>
<td>2472.57</td>
<td>3.28</td>
<td>0.072</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>291</td>
<td>763.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Latency to oviposition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td></td>
<td>0.168</td>
<td>3</td>
<td>296.95</td>
<td>0.38</td>
<td>0.768</td>
</tr>
<tr>
<td>Block*thorax</td>
<td></td>
<td></td>
<td>4</td>
<td>32.58</td>
<td>1.59</td>
<td>0.178</td>
</tr>
<tr>
<td>High/Low(thorax)</td>
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<td>1</td>
<td>43.01</td>
<td>0.05</td>
<td>0.815</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td></td>
<td>293</td>
<td>782.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean egg number</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
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<td>0.208</td>
<td>3</td>
<td>60.52</td>
<td>2.96</td>
<td>0.034</td>
</tr>
<tr>
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<td></td>
<td>4</td>
<td>32.58</td>
<td>1.59</td>
<td>0.178</td>
</tr>
<tr>
<td>High/Low(C2)</td>
<td></td>
<td></td>
<td>1</td>
<td>142.94</td>
<td>6.99</td>
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<tr>
<td>Female thorax</td>
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<td></td>
<td>291</td>
<td>21.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td></td>
<td>293</td>
<td>21.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean egg number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
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<td>0.194</td>
<td>3</td>
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<td>0.828</td>
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<tr>
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<td></td>
<td>4</td>
<td>6.35</td>
<td>0.30</td>
<td>0.877</td>
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<tr>
<td>High/Low(thorax)</td>
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<td></td>
<td>1</td>
<td>2.13</td>
<td>0.10</td>
<td>0.751</td>
</tr>
<tr>
<td>Female thorax</td>
<td></td>
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<td>1</td>
<td>134.59</td>
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<tr>
<td>Error</td>
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<td></td>
<td>293</td>
<td>21.76</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Mean C2 teeth number counts (A) and thorax length measurements (B) for males mating 0, 1 or 2 times. Error bars represent the SE. P-values were as follows: C2: $p=0.875$; Thorax length: $p=0.707$. Numerals= $n$
Figure 2. Relationship between the residual log of latency to copulate and (A) C2 tooth numbers and (B) male thorax lengths (see Table 3). The residuals were taken from the significant effect of block on log latency. The mean latency value was added to all residuals.
Figure 3. The effect of male category (‘high’ and ‘low’ in terms of C2 teeth numbers and thorax lengths) on latency to copulate. Error bars represent the SE. P-values were as follows: C2: $p = 0.221$; Thorax length: $p = 0.308$. Numerals = $n$. 
Figure 4. Relationship between the residual log of duration of copulation and (A) C2 teeth counts and (B) male thorax lengths (see Table 3). The residuals were taken from the significant effect of block on log duration. The mean duration value was added to all residuals.
Figure 5. The effect of male category (‘high’ and ‘low’ in terms of C2 tooth number and thorax lengths) on duration of copulation. Error bars represent the SE. P-values were as follows: C2: $p=0.534$; Thorax length: $p=0.198$. Numerals= $n$. 
Figure 6. Relationship between residual latency to oviposit and (A) C2 teeth counts and (B) male thorax length (see Table 5). The residuals were taken from the significant effect of block on latency to oviposition. The mean latency to oviposit was added to all residuals.
Figure 7. The effect of male category (‘high’ and ‘low’ in terms of C2 tooth number and thorax lengths) on latency to oviposit. Error bars represent the SE. P-values were as follows: C2: $p=0.072$; Thorax length: $p=0.815$. Numerals= $n$. 
Figure 8. Relationship between residual mean number of eggs laid at the first oviposition event and (A) C2 teeth counts and (B) male thorax lengths (see Table 5). The residuals were taken from the significant effect of block on mean number of eggs laid at the first oviposition event. The mean number of eggs laid at the first egg-laying event was added to all residuals.
Figure 9. The effect of male category (‘high’ and ‘low’ in terms of C2 tooth number and thorax lengths) on mean number of eggs laid. Error bars represent the SE. P-values were as follows: C2: $p=0.255$; Thorax length: $p=0.751$). Numerals= $n$. 
CHAPTER 2

Different secondary sexual traits reveal a common genetic quality of males

Alison F. Cooperman, Michal Polak, Christopher S. Evans & Phillip W. Taylor

(submitted to Behavioral Ecology)

Short Running Title: Male genetic quality

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ABSTRACT

A key unresolved question in sexual selection research is whether different sexual traits are developmentally independent or are integrated manifestations of a common genetic property. If multiple traits of individuals are condition-dependent expressions of a common genetic property conferring quality, then predictable associations should exist in the expression of traits across genotypes. Here we present evidence for positive covariation between morphological (sex comb size) and behavioral (courtship song) sexual traits among genetic lines of *Drosophila bipectinata* Duda recently extracted from the wild. We detected significant body size-independent differences in comb size among lines. Replicate lines exhibiting relatively high and low values of comb size were then subjected to analyses of courtship song. The high sex comb lines exhibited shorter mean burst period and shorter mean burst duration than low sex comb lines. Our results demonstrate a genetic association between condition-dependent traits, and suggest that these traits reveal overall genetic quality.
INTRODUCTION

Several leading models describing the evolution of secondary sexual traits require that sexual trait expression covaries positively with overall genetic ‘quality’ of individuals (Zahavi, 1975; Kodric-Brown and Brown, 1984; Grether, 1996; Andersson et al., 2002; Kokko et al., 2003). Genetic quality may be envisioned as a genotype’s ability to acquire limiting resources from the environment, so that individuals of higher quality come to accumulate a greater pool of resources (i.e., condition) available for allocation among competing physiological functions (Rowe and Houle, 1996; Bonduriansky and Rowe, 2005).

An important prediction of the hypothesis that variation in sexual trait expression reveals differences in overall genetic quality, but one that has received little attention, concerns covariation in expression among different condition-dependent sexual traits of individuals. If the different traits are expressions of a common underlying genetic property conferring quality (e.g., Møller and Pomiankowski, 1993; Johnstone, 1996; Møller and Mousseau, 2003), and given sufficient variation in genetic quality or condition, then there should exist a positive relationship in expression of different condition-dependent sexual traits (Kodric-Brown and Brown, 1984; Johnstone, 1995b; Candolin, 2003), even if the traits in question are very different in kind (e.g., morphological vs. behavioral). Houle (1991) provided theoretical rationale for this prediction, by showing that if certain reasonable assumptions concerning the genetic architecture of resource acquisition are met, the equilibrium additive genetic covariation among condition-dependent traits can be positive.
The few empirical studies that have tested for covariation among secondary sexual traits have focused on the phenotypic level, and have yielded mixed support for this prediction (e.g., Andersson et al., 2002; Møller and Petrie, 2002; Jawor and Breitwisch, 2004; Moczek et al., 2004). Noteworthy, therefore, is a study of guppies (Poecilia reticulata), showing no consistent trend toward positive genetic covariation among different ornamental traits of males, suggesting that collectively these traits do not reveal a common genotypic property of males (Brooks and Endler, 2001). Similarly, Bonduriansky and Rowe (2005) found that out of all possible genetic correlations between four sexually selected, condition dependent traits in the piophilid fly, Prochyliza xanthostoma, only one correlation was significantly positive. Interestingly, this significant correlation occurred between head length and antenna length, the two traits exhibiting strongest condition dependence.

Here we test for an association arising because of genotypic effects between two very different condition-dependent sexual traits of male Drosophila bifectinata: sex comb size and elements of courtship song. The sex comb consists of stout teeth arranged in rows on the first and second tarsal segments of the male front legs (Figure 1A). Polak and Starmer (2005) have shown that a 4°C increase in developmental temperature increases comb fluctuating asymmetry (a putative measure of developmental instability), and reduces both comb tooth number and adult body size, suggesting that comb expression is condition-dependent. Comb size (i.e., tooth number) is heritable and under positive sexual selection in natural populations in northeastern Australia (Polak et al., 2004).
Courtship songs in *Drosophila* are the product of wing beats produced by the insect’s underlying flight musculature (Ewing, 1979), and as such have the potential to exhibit condition dependence, and hence to reveal differences in male genetic quality (e.g., Hoikkala and Isoherranen, 1997; Hoikkala et al., 1998). *Drosophila* song consists of bursts (trains) of sound pulses (Figure 1B), and studies have shown that certain features of this structure can be direct targets of mate choice. For example, in *D. montana*, males producing songs with shorter pulses and higher carrier frequency are preferred by females (Aspi and Hoikkala, 1995; Ritchie et al., 1998; Hoikkala and Suvanto, 1999), and produce progeny with elevated survival rates (Hoikkala et al., 1998). Although not previously studied in *D. bipectinata*, there is also compelling evidence for condition dependent expression of some song features in other species. In both *D. montana* and *D. littoralis*, overwintering, field-caught males show high repeatability for pulse length, suggesting condition-dependent variation of this trait (Hoikkala and Isoherranen, 1997). Pulse number (a determinant of burst duration) also shows evidence of condition-dependent expression (as revealed by significant repeatability following cold stress), and exhibits significant heritability in laboratory and field populations of *D. montana* (Aspi and Hoikkala, 1993; Hoikkala and Isoherranen, 1997). In *D. mojavensis*, burst duration differs geographically, and parallels differentiation in epicuticular hydrocarbons which serve as contact pheromones during courtship (Etges et al., 2006).

Here we examine variation across genotypes within an Australian population of *D. bipectinata* in burst duration (BD) and burst period (BP) in the context of variation in sex comb size.
We extracted genotypes (iso-female lines) from a natural population, and chose lines with largest and smallest mean sex comb size for analysis of male courtship song. If degree of investment into the sex comb reflects a genetic quality shared at least in part with that of song, we made the specific prediction that individuals from lines developing larger combs should on average express shorter BPs and BDs. This prediction is reasonable because since shorter values of BD and BP result in a higher rate of burst production, BDs and BPs should reflect male stamina and possibly physiological recovery rate.

Because some song components were significantly correlated with comb size, we tested for song differences among lines while factoring out sex comb size variation in order to control for scaling between these traits. This correction is necessary for establishing whether song differences reveal properties other than scaling with sex comb size (the trait used to originally choose the lines). Figure 2A illustrates the hypothetical situation wherein differences between observed song means (\( \bar{Y}_1 \) and \( \bar{Y}_2 \)) occur entirely as a result of scaling (and see Cotton et al., 2004, p. 781). We envision that such scaling could arise because a small subset of loci coding specifically for the sex comb exerts pleiotropic effects on song attributes, and not because of generalized genotypic effects. Thus, once adjusted to a common sex comb size (\( \bar{X}_o \)), line means (\( \hat{\bar{Y}}_1 \) and \( \hat{\bar{Y}}_2 \)) cease to differ. In Figure 2B, however, song means differ despite the correction. We envision that this outcome could arise if the condition dependent sexual traits reflect a common inherent property of the lines: the effects of loci throughout the genome that collectively contribute to the genetic component of condition, or overall genetic quality.
MATERIALS AND METHODS

Isofemale lines

A total of 32 lines were established, each from a single copulating pair of *D. bipectinata* captured in the field. Individual pairs were gently aspirated directly into 35 ml vials with food from the surface of exposed flesh of jackfruit, *Artocarpus heterophyllus* Lam. (Moraceae), from 11 - 14 January 2004 at the Cape Tribulation Farmstay, northeastern Queensland, Australia. Pairs were captured between 5:15 – 6:30 AM, coinciding with the narrow period of peak mating activity for this species (Polak et al. 2004). Each newly captured female was allowed to oviposit for 3 d into a 35 ml food vial, and transferred to a fresh vial for another 3 d, for a total of two vials per line. The food consisted of 1.7g instant *Drosophila* medium (Carolina Supply Co.), 8 ml water, and 1 ml of crushed banana mixed with live yeast slurry. A general stock of flies was established from ca. 50 field-caught pairs. On January 16, flies were brought to a laboratory at Macquarie University, Sydney, Australia, and maintained in an environmental room at 24–26°C and 60-70% RH, and at a 12 h light: 12 h dark photoperiod (lights came on and off at 6:00 AM and 6:00 PM, respectively).

Sex comb size and thorax length (estimate of body size) were determined for 8 – 10 *F₁* flies emerging from each replicate vial per isofemale line. Flies were killed with ethylene dichloride fumes, and their foretarsi placed on the surface of double-sided transparent tape. For each male, the numbers of teeth in the sex comb segments on each foretarsus were counted under an Olympus SZX12 microscope, and thorax length was measured using an ocular micrometer of a WILD M4A microscope. We define ‘comb size’ as the number of teeth summed across the first and second segments of the comb.
(i.e., C1 + C2) (Figure 1A); C1 and C2 constitute the major elements of the comb, and are positively correlated genetically (Polak et al., 2004).

We used an ANCOVA, with line and vial (nested within line) as factors, and thorax length as the covariate, to test for comb size differences among lines. Four test lines were chosen for acoustic analysis: two lines each exhibiting relatively high and relatively low mean comb size. Lines were cultured for another generation under conditions specified above, and F2 flies from each line were characterized for comb size as above. ANCOVA tested whether the differences in comb size between lines persisted among the F2 flies (i.e., exhibit cross-generational stability in expression). F2 and F3 flies were subjected to acoustic analysis.

**Recording fly song and behavior**

The courtship songs of *D. bipectinata* are produced at very low amplitude, so all audio- and video-recordings were made in a sound-attenuating chamber (2.38 m wide x 2.15 m high x 2.38 m deep; Amplisilence S.p.a.), lined with 10 cm ‘Sonex’ foam baffles (Illbruck Inc) on the side walls and 15 cm baffles on the ceiling. To further reduce external noise, a double-walled rectangular sound-attenuating box was constructed. The test arena was mounted within this box and illuminated by a 15-Watt incandescent lamp.

Flies were confined within a cylinder (diameter 22 mm, height 4 mm) of soft clear plastic and a floor of fine PVC-coated fibreglass mesh (Cyclone MINIwave™ 0.25 mm yarn, 7.2 x 12 strands/cm). The top of the cylinder was sealed with a glass microscope slide, above which was mounted a video camera (Panasonic WV-BP550) with a wide-angle lens. A Sennheiser MKH-40 microphone was clamped immediately below the
arena floor, facing upward. Flies were inserted through a 40 mm-long tube (3 mm internal diameter) joined to a port in the arena wall.

Video signals were recorded on a digital VCR (Sony DSR-20P) in PAL DVCAM format (resolution 576 x 720 pixels, 50 fields/s). The microphone was connected to a Behringer MIC-100 pre-amplifier, which also provided 48V phantom power. To obtain additional gain, the output signal was then fed through an amplifier/mixer (Behringer Eurorack MX602A) which also converted the balanced microphone output to an unbalanced line-level signal suitable for the digital VCR. Audio levels were metered at every step in the recording pathway to ensure that clipping did not occur. Sound was digitized at 44.1 kHz, 16 bits. This system has a flat frequency response (+1 dB) from 50 Hz – 20 kHz, with no wow or flutter. The video and sound tracks were synchronized using a common time code.

Sound recordings were made between 5:30 and 8:00 am. Female subjects were 3 d old and had been separated from males for at least 24 h. Flies were kept in the dark inside a box within the environmental room overnight until immediately prior to testing; peak courtship activity of this species in the field begins at first light, and runs to ≈ 1 h after sunrise (Polak et al. 2004). A female was transferred with an aspirator from her holding vial to the arena through a tube. A barrier of clear polyacetate film was inserted through a slit in this tube close to the arena wall to prevent her escape. A male fly was then aspirated into the tube, and the open end of the tube was plugged with a dowel. After 5 min of acclimation, the barrier was removed and the dowel was gently pushed into the tube, thus encouraging the male to enter the arena. No fly of either sex was anesthetized on the morning of its use in recordings. Recordings lasted 13 minutes for each male.
During recording sessions, we watched interactions on an external video monitor and listened to the recorded song with headphones. Video time-code was continuously displayed to track recording duration. The temperature of the sound-attenuating chamber was noted at the beginning and end of each recording.

**Analyses of song recordings**

Recordings from the digital Sony VCR were transferred to a computer (Macintosh G4, Apple Computer) via IEEE 1394 ‘firewire’, using iMovie software. Original DV files of 2-5 GB each were then MPEG-4 compressed at 80% quality to generate QuickTime files of typically 0.5GB using Cleaner software 6 (Discreet). The video stream was de-interlaced and noise-reduced, but the sound track was left uncompressed to avoid the introduction of artifacts. Sections containing multiple bouts of male courtship were then isolated and saved as self-contained Quicktime movies. Copies of the sound track were created as mono audio (.aif) files. These were then further processed and analyzed using Canary v. 1.2.4 (Cornell Bioacoustics).

To remove background sounds that masked songs in the time domain, all audio files were first high-pass DFT filtered in Canary, completely attenuating frequencies below 80 Hz. Preliminary analyses had revealed that most of the noise was within the filter stop-band; later checks of song fundamental frequency confirmed that no song components were affected by the filter.

Within the 13 minutes of video recording, males typically engaged in multiple bouts of courtship. A bout of courtship began when a male engaged in courtship, and ended when he mounted or when the pair separated by > 2.5 body lengths. During
courtship, males produce bursts (or trains) of sound pulses. We measured burst duration (BD) and burst period (BP) from oscillograms generated by Canary (Figure 1B) for songs produced during a male’s first courtship bout (and see Crossley (1986) for similar terminology). Song measurements were made without knowledge of the identity of the singing male (i.e., to which line it belonged). For some courtship bouts, song was of insufficient amplitude and therefore not clearly discernable because males were too far from the microphone. In these cases, subsequent bouts were sampled. The courtship bout from which temporal measurements were obtained (1, 2, 3 or 4) for a given male was coded in preliminary analyses to check whether bout explained variation in male courtship (see next section). Burst duration and BP were distinguished according to the following behavioral phases of courtship. During ‘distant pursuit,’ males chase females from 2.5 – 1.5 body lengths away, whereas during ‘close pursuit’ males chase females from a distance of ≈ 2 body lengths to the tip of her wings. During ‘near contact,’ females are typically stationary and males court from a distance of 1 body length to a head-under-her-wings position. When mounting, a male climbs or lunges on top of a female, grasps her with his foretarsi (at which point the combs come into contact with the female’s abdomen), and curls his abdomen downward. A successful mounting results in copulation. A mounting, whether successful or not, ends a bout of courtship. We distinguished song BP and BD according to these 3 courtship phases in order to refine our analysis of song variation. Males produce a long song (many pulses (e.g., 25) per burst) during distant pursuit, whereas during close pursuit and near contact, males predominantly sing a short song (few [e.g., 3] pulses per burst) (Crossley, 1986). It was
important to distinguish these song types because song parameters, such as the BP, can vary significantly between them (Crossley, 1986).

The song of a total of 79 males ($n=38$ and 41 from high and low comb size lines, respectively) was analyzed. However, we were unable to acquire BP and BD for all phases of courtship for all males, either because males did not sing during a particular phase, or because song was of insufficient amplitude to be confidently discerned (see above). Moreover, distant pursuit occurred especially infrequently, so sample sizes are least for this phase of courtship. Multiple BPs and BDs for a given phase were measured per courtship bout of a male, and a mean for that male was calculated and entered into MANOVAs, below. Number of males, and mean number of measurements for a given song trait made per male, are reported.

**Song differences among lines**

Multiple analyses of covariance (MANCOVAs) with comb size as the covariate were conducted to discriminate between high and low sex comb lines with respect to BP and BD in the different courtship phases. Although the comb size covariate was significant only for burst duration (see Results), comb size was entered as a continuous variable in all analyses for consistency among traits. In a first MANCOVA, we tested for effects of line on four dependent variables: BD and BP during near contact and close pursuit. A second MANCOVA tested for line effects on these same traits, but during distant pursuit. Separate MANCOVAs were necessary because distant pursuit occurred less frequently than either the close pursuit or near contact courtship phases. Roy’s greatest root $F$ statistic was used to derive statistical conclusions in all MANCOVAs.
Squared canonical correlations ($r^2$) are reported to describe between-line variation collectively explained by the response variables. ‘Protected’ univariate ANCOVAs (Scheiner, 1993) were conducted subsequent to detecting significant line effects with either MANCOVA. The homogeneity of slopes assumption across replicate lines was verified by testing for an interaction between line and comb size; in no case was this interaction significant. Distributions of residuals were visually inspected to check for normality and for constancy of error variances across groupings. The influence of conspicuous outliers was quantitatively assessed by the DFFITS statistic (SAS, 2002).

The addition of male thorax length, female thorax length, temperature, and bout, as covariates in preliminary MANCOVAs revealed that none of these variables had significant effects on the dependent variables; these variables were thus excluded from the reported analyses. Significant effects of temperature on *Drosophila* song parameters are known (e.g., Ritchie and Kyriacou, 1994; Noor and Aquardo, 1998), and their absence here may be attributable to the narrow range of temperatures recorded in the sound-attenuating chamber (range: 25-27.5°C, $\overline{X} \pm SD: 26.1 \pm 0.85°C$).

**RESULTS**

**Choosing high and low sex comb lines**

ANOVA, with line and vial (nested within line) as factors, and male thorax length as the covariate, revealed significant differences in comb size (i.e., C1+C2, Figure 1A) among the set of lines originally surveyed ($F_{27,87} = 1.76, p = 0.03$). Four lines were chosen, based on mean comb size, to represent the experimental lines: two lines with the lowest mean comb size, and two lines with the highest mean comb size (Table 1).
A second ANCOVA on F₂ flies, with line and male thorax length as the covariate, revealed that the comb size differences among high and low lines persisted in the subsequent generation \( (F_{3,71} = 31.31, p < 0.0001) \). The high lines maintaining a higher comb size than the low lines (Table 1). Thus, these results demonstrate cross-generational stability in the expression of trait differences, and confirm previous work on the genetic basis of variation in sex comb size (e.g., Polak et al., 2004).

**Testing for song differences among lines**

MANCOVA, with comb size as the covariate, on BP and BD during close pursuit and near contact variables, revealed a significant overall effect of line \( (F_{4,25} = 4.14, p = 0.010, r^2 = 0.40) \). Protected ANCOVAs were therefore justified (Scheiner 1993). These analyses revealed no effect of line on near contact BP \( (F_{3,53} = 2.50, p = 0.069) \), near contact BD \( (F_{3,61} = 0.34, p = 0.80) \), and close pursuit BD \( (F_{3,41} = 1.40, p = 0.26) \). Least-square means for all song traits are provided in Table 2. Only in the case of close pursuit BP was the line effect significant. However, three outliers with considerable influence were detected at the high end of the close pursuit BP distribution. The removal of these outliers from the ANCOVA normalized the residuals, but eliminated the significant effect of line on close pursuit BP \( (F_{3,35} = 0.42, p = 0.74) \) (Table 2). In an alternative approach using the full data set with these outliers included, we performed an ANCOVA on rank-transformed (Conover and Iman, 1981) close pursuit BP values, which normalized the data, and likewise found no effect of line on close pursuit BP \( (F_{3,38} = 1.52, p = 0.23) \). The comb size covariate was not significant in any of the above ANCOVAs \( (p \text{ values: 0.26 - 0.97}) \).
The second MANCOVA revealed a strong effect of line on the song variables expressed during distant pursuit ($F_{3,15} = 15.39, p < 0.0001, r^2 = 0.75$). The comb size covariate was significant in the analysis of BD ($F_{1,17} = 5.36, p = 0.03$), but not for BP ($p = 0.095$) (Table 2). Protected ANCOVAs showed significant line effects on both BP ($F_{3,16} = 5.82, p = 0.0069$) and BD ($F_{3,17} = 4.47, p = 0.017$) (Figure 3). Thus, genotypes that develop a large body size-specific sex comb, also express a shorter burst period and burst duration, but only during distant pursuit courtship.

DISCUSSION

Our initial screening of 32 genetic isolates of *D. bipectinata* extracted from a natural population in northeastern Australia revealed significant among-line variation in sex comb tooth number (comb size), corroborating the results of a previous study showing significant heritable variation underlying comb size in the same population (Polak et al., 2004). We used replicate lines from either extreme of the sex comb size distribution, and showed that these differences persisted through to F$_2$ progeny, thus confirming their genetic basis.

The most intriguing result of the present work is that these replicate lines chosen from either extreme of the comb size distribution exhibited the predicted difference in two components of male courtship song. This finding suggests integration among these very different classes of secondary sexual traits. By using such maximally diverged lines in regard to sex comb size, we effectively increased the sensitivity of our assays for detecting correlated differences in the second trait. Specifically, males from the high sex comb lines produced songs with significantly shorter mean burst period (BP) and mean burst duration (BD) during the distant pursuit phase of courtship than did males from the
genetic lines developing smaller combs. The reason for why these differences are restricted to distant pursuit courtship song is unknown. It may be, for example, that song production during this phase of courtship is relatively more expensive energetically in order to accommodate the greater distance the signal must travel, thus only revealing quality differences among competing genotypes at this phase. Indeed, during distant pursuit males produce bursts of song that consistently contain more pulses per burst than during other phases of courtship (Crossley, 1986). If more pulses per burst and a shorter latency between bursts require greater energy expenditure and/or shorter recovery times, then song production during distant pursuit may be pushing males especially close to their physiological limits, thus revealing quality differences among them at this phase.

Because both the sexual traits we measured are likely to be condition dependent (see Introduction), their coordinated expression at the genotypic level provides support for a key hypothesis of 'good genes' sexual selection theory, namely, that degree of sexual trait expression reveals variation in overall quality (i.e., condition) among genotypes within a population (Kodric-Brown and Brown, 1984; Andersson, 1994; Johnstone, 1995a; Kokko et al., 2003). Thus, sex comb size and elements of courtship song appear to be acting as ‘redundant’ (Møller and Pomiankowski, 1993), or at least overlapping, signals of male genetic quality (Johnstone, 1996; Candolin, 2003). It follows that comb size and song may be acting as a suite of traits from which females may acquire reinforcing information regarding the quality of a potential mate. However, although males with larger combs enjoy a higher probability of copulating in at least one field population (Polak et al. 2004), whether either of these traits in *D. bipectinata* are the direct targets of female choice remains to be explored.
The present study is unique because it reports an association across genotypes between two very different sexual traits, one being morphological and the other behavioral. A genetic origin of this coordinated expression is further supported by our having reared the lines under common environmental conditions, a procedure that minimized the possibility of environmentally induced covariation among the traits, which could arise from shared sensitivity of independent developmental pathways to a common environmental agent(s) (Klingenberg, 2003). The likely involvement here of largely separate pathways is suggested by the different nature of these traits: the sex combs, which occur on the anterior-ventral portion of the front tarsi of males, are modified mechanosensory bristles (Kopp and True, 2002), whereas courtship songs are the result of wing movements generated by the neuromusculature within the thoracic cavity (reviewed in Gleason, 2005).

We also considered the possibility that the observed associations arose because of a direct interaction between the different developmental networks producing these traits, a recognized cause of covariation among traits of individuals (e.g., Cheverud, 1982; Nijhout and Emlen, 1998). Under such a 'local effects' model, the observed differences in sex traits among lines would merely reflect scaling between the traits, and not differences in overall genetic quality (Figure 2). Such scaling could arise in cases where natural allelic variation at a relatively small subset of developmental loci of the sex comb were to differentially influence the development of song-producing neuromuscular systems. The existence of such ‘local effects’ is feasible because in at least one case (burst duration during distant pursuit) song was significantly correlated with comb size at the individual level. Thus, we accounted for the scaling possibility by correcting song features for inter-
individual differences in comb size using analysis of covariance. This approach of statistical control was applied to factor out any effects of scaling between song and sex comb size (Figure 2). A nonsignificant line effect in our analyses of covariance would have supported the 'local effects' hypothesis, as occurred in a previous study of the relationship between a measure of developmental instability and mating behavior (Polak and Stillabower, 2004), an outcome receiving its own theoretical support (Klingenberg and Nijhout, 1999). In the present study, however, strong differences in song structure between the genetic isolates persisted despite the correction. Hence, our data support the hypothesis that these different secondary sexual traits of *D. bipectinata* tap a general property of the genotypes we surveyed, namely, that which confers overall genetic quality related to the acquisition of condition.
REFERENCES


Table 1. Mean peg number (i.e., comb size) ± SE (n males) in F$_1$ and F$_2$ flies from lines subjected to acoustic analysis.

<table>
<thead>
<tr>
<th>Line</th>
<th>Generation</th>
<th>F$_1$</th>
<th>F$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td></td>
<td>27.0 ± 0.43 (20)</td>
<td>28.38 ± 0.43 (20)</td>
</tr>
<tr>
<td>H2</td>
<td></td>
<td>26.2 ± 0.47 (20)</td>
<td>27.16 ± 0.45 (17)</td>
</tr>
<tr>
<td>L1</td>
<td></td>
<td>24.4 ± 0.47 (20)</td>
<td>22.88 ± 0.41 (20)</td>
</tr>
<tr>
<td>L2</td>
<td></td>
<td>22.9 ± 0.47 (20)</td>
<td>24.04 ± 0.44 (19)</td>
</tr>
</tbody>
</table>
Table 2. Least-square mean ± SE burst period (BP) and burst duration (BD) in the three courtship phases of *D. bipectinata*. Number of males and mean number of measurements per male are given in parentheses for each trait.

<table>
<thead>
<tr>
<th>Line</th>
<th>Courtship phase/song trait</th>
<th>H1</th>
<th>H2</th>
<th>L1</th>
<th>L2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near contact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td></td>
<td>0.323 ± 0.054</td>
<td>0.321 ± 0.054</td>
<td>0.474 ± 0.046</td>
<td>0.332 ± 0.067</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(16, 18.3)</td>
<td>(12, 12.4)</td>
<td>(17, 13.3)</td>
<td>(13, 8.9)</td>
</tr>
<tr>
<td>BD</td>
<td></td>
<td>0.114 ± 0.018</td>
<td>0.0997 ± 0.018</td>
<td>0.123 ± 0.016</td>
<td>0.122 ± 0.024</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(19, 16.4)</td>
<td>(14, 13.4)</td>
<td>(19, 14.3)</td>
<td>(14, 9.4)</td>
</tr>
<tr>
<td>Close pursuit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP*</td>
<td></td>
<td>0.358 ± 0.045</td>
<td>0.353 ± 0.044</td>
<td>0.421 ± 0.050</td>
<td>0.393 ± 0.060</td>
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<tr>
<td></td>
<td></td>
<td>(12, 7.9)</td>
<td>(11, 6.4)</td>
<td>(8, 6.8)</td>
<td>(9, 4.5)</td>
</tr>
<tr>
<td>BD</td>
<td></td>
<td>0.180 ± 0.040</td>
<td>0.097 ± 0.042</td>
<td>0.150 ± 0.048</td>
<td>0.221 ± 0.055</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14, 8.9)</td>
<td>(11, 6.1)</td>
<td>(8, 7.3)</td>
<td>(10, 4.5)</td>
</tr>
<tr>
<td>Distant pursuit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BP</td>
<td></td>
<td>0.260 ± 0.14</td>
<td>0.383 ± 0.18</td>
<td>1.27 ± 0.19</td>
<td>0.873 ± 0.16</td>
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<td></td>
<td></td>
<td>(7, 4.4)</td>
<td>(4, 4.0)</td>
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<td>(7, 3.4)</td>
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<tr>
<td>BD</td>
<td></td>
<td>0.106 ± 0.025</td>
<td>0.0956 ± 0.033</td>
<td>0.243 ± 0.030</td>
<td>0.218 ± 0.029</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7, 4.4)</td>
<td>(4, 3.8)</td>
<td>(4, 3.8)</td>
<td>(7, 3.3)</td>
</tr>
</tbody>
</table>

* Means for this trait do not include outliers (see text for details).
Figure 1. (A) SEM of the male sex comb in *D. bipectinata* (486x). In this specimen, there are 6 and 8 teeth in the first (C1) and second (C2) comb segments, respectively; distal end of the tarsus is to the right. (B) Oscillogram of male courtship song showing burst duration (BD) and burst period (BP).
Figure 2. Two scenarios accounting for differences in condition dependent trait B (in our case song) among lines divergent for sex comb size; four individuals per line are represented. (A) Differences in trait B arise entirely because of scaling with comb size (trait A). Analysis of covariance in this case reveals no line effects on trait B. (B) The two traits scale with different intercepts, and analysis of covariance reveals significant line effects on trait B expected to reflect inherent quality differences among the genotypes related to the acquisition of somatic condition.
Figure 3. Least squares mean duration of the burst period and burst duration during distant pursuit in male *D. bipectinata* for high (H) and low (L) sex comb genetic lines extracted from a natural population. Error bars represent ± 1 standard error.
SUMMARY

This thesis concerns the role of the male sex comb of *Drosophila bipectinata* in sexual selection. The accumulation of data generated from the projects conducted for the completion of this Master’s degree concluded the following:

Project 1 (Chapter 1):

1. The aim of project 1 was to test potential mechanisms of sexual selection which might attest for the differential mating success of male *D. bipectinata* with larger sex combs observed in a field population (Polak et al. 2004). The results show that male *D. bipectinata* with more sex comb teeth are not more likely to mate than males with fewer teeth per sex comb. Male body size, however, did correlate with the occurrence of mating on the first day of experimentation, such that larger males were less likely to achieve copulation than smaller males. There was no significant effect of size on mating success on the second day of experimentation. Tests of repeatability verified that the behavior of males was inconsistent (i.e., not repeatable) over the two days of experimentation despite the fairly uniform experimental design and homogenous environment provided. Slight deviations in the assay between the first and second days of observations were advanced as potential explanations for this discrepancy in behavior across days.

2. Of the males that were observed mating, their sex comb tooth number was not correlated with their latency to copulate, or the duration of that copulation. Similarly, male body size did not significantly influence the expression of either latency to, or duration of, copulation. The tests of these predictions do not
support the hypothesis that the mating success of male *D. bipectinata* in the field can be attributed to these particular mechanisms of intra-sexual competition. Confidence in the data is low, as the assay chosen to detect these behaviors may not have sufficiently emulated natural conditions. Thus, the possibility that latency to, and duration of copulation are mechanisms that distinguish successful males from unsuccessful males in the field does remain a feasible possibility worthy of further exploration.

3. Neither male sex comb number nor body size were associated with either the latency of the female they paired with to lay eggs, or the number of eggs laid at that time. These results failed to support the prediction that female cryptic choice, as reflected in a latency to oviposit and eggs laid at that first egg-laying event, is occurring in this system. Again, a better prediction of this hypothesis involves a comparison of female oviposition and egg-laying rate with multiple males who differ in sex comb size. Quantifying the viability of the offspring produced by the pairs of flies is just one example of another potential mechanism of female cryptic choice that was not examined here. Therefore, whereas the evidence here is not consistent with female cryptic choice, more tests of the hypothesis would need to be conducted in order to rule out its occurrence in this system.

**Project 2 (Chapter 2):**

1. The pursuit of a genetic association between sex comb tooth number and some song parameters in project 2 was meant to test Rowe and Houle’s (1996) model
describing how such a situation, where multiple condition-dependent traits covary positively, can exist. These results would then support the ‘good genes’ models of sexual selection. The creation of multiple genetic (iso-female) lines provided evidence of significant variation in sex comb tooth number among the lines surveyed. This supports Polak et al.’s (2004) work revealing a genetic (i.e., heritable) basis of sex-comb tooth number, and makes feasible the potential for a genetic association between other traits.

2. Males from genetic lines exhibiting relatively high sex comb tooth numbers, independent of body size, also expressed significantly shorter burst durations (BD) and burst periods (BP - both components of courtship song) during the distant pursuit phase of courtship. Males from genetic lines averaging fewer sex teeth per comb, then, had significantly higher values of BD and BP during distant pursuit. These results persist after correcting the data for sex comb size, the trait that was used to initially differentiate the lines.

3. These findings suggest that 1) there is positive covariation between 2 condition dependent sex traits and 2) this association is mediated by a shared set of loci that contribute to the general condition (i.e., quality) of the organism. The results corroborate the ‘good genes’ models of sexual selection, which predict that variation in trait expression is attributable to differences in the quality of the organism harboring the traits.
FUTURE DIRECTIONS

While the 1st project aimed at answering why males with larger combs were more successful in the field did not accomplish this, the results from project 2 evoke an answer for their success: sex-comb size is associated with courtship song, and both are capable of signaling quality to potential mates. And although Polak et al.’s 2004 field data opens up the possibility that females may have been receptive to those signals by mating more often with certain males (those having more combs), there is no evidence as of yet to show that sex comb tooth number or song parameters are targets of female preference in *D. bipectinata*. Ideas on how to approach accomplishing this feat include:

1. Manipulations of both sex combs and courtship songs. These would be powerful strategies to employ in order to illuminate the role of these sex traits in mate choice, beyond their function in species recognition. For example, playback experiments, where song parameters are modified (i.e. sped up or slowed down), could be played to females whose behaviors, when exposed to different song types, are quantified.

2. Removing sex teeth in the sex combs and pairing those males with females, pre and post- manipulation would be one way to gage the degree of attractiveness of the sex combs. Even if combs were not found to be direct targets of female choice, they could at least be considered a by-product or correlated response to selection on some other trait. The genetic association uncovered in project 2 permits this possibility.
As with most scientific inquiries, the answers to the questions initially posed about the *D. bipectinata* system leave much to be desired; if latency and duration of copulation do not explain the mating success of males with larger combs in a field population (as observed by Polak et al. 2004), what does? Were the assay of this experiment modified to emulate a more realistic scenario (i.e., multiple males and non-virgin females on fruit at dawn), would the results have suggested a larger role of these intra-sexual mechanisms? If males from genotypes exhibiting relatively larger combs sung faster than males from genotypes exhibiting smaller combs, how else might they differ? How, exactly, are sex combs and song parameters related to one another, in other words, what are the ‘good genes’ and what other life history characters do they influence? These questions are particularly pertinent to studies of sexual selection theory, and the *D. bipectinata* species has proven itself an ideal model system to explore these issues further.