ABSTRACT

Title of Dissertation:	TRADE-OFFS WITHIN AND BETWEEN SEXUAL TRAITS IN STALK-EYED FLIES (DIPTERA: DIOPSIDAE)
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The allocation of limited resources to competing body parts during development may affect both the absolute and relative sizes of physical traits, creating potentially dramatic consequences for the evolution of morphology. While negative correlations between the sizes of body parts ("trade-offs") arising from the distribution of finite resources have long been anticipated, empirical support is relatively rare. In this study, I use two related species of stalk-eyed flies that differ in morphology to investigate whether eye-span exaggeration results in trade-offs with other body parts. One species, *Cyrtodiopsis dalmanni*, is sexually dimorphic, with males having exaggerated eye-span while the other, *C. quinqueguttata*, is sexually monomorphic with both sexes having approximately equivalent and relatively unexaggerated eye-span. I utilize complementary approaches including artificial selection, application of exogenous juvenile hormone, and diet manipulation to alter the absolute and relative length of the eye-stalks in order to reveal correlated changes in other physical traits. The results of

these experiments suggest that exaggerated eye-span in male *C. dalmanni* is accompanied by a decrease in two other features of head morphology, eye-bulb size and eye-stalk width, as well as compromised testis growth and sperm production. No trade-offs were observed in females of either species or male *C. quinqueguttata*. These results are consistent with hormonally-mediated trade-offs arising from the allocation of limited resources to an exaggerated trait and suggest that those trade-offs may act as costs of developing exaggerated eye-span.

TRADE-OFFS WITHIN AND BETWEEN SEXUAL TRAITS IN
STALK-EYED FLIES (DIPTERA: DIOPSIDAE)

By

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TABLE OF CONTENTS

LIST OF FIGURES

v

INTRODUCTION

I. BACKGROUND

"…I see hardly any way of distinguishing between the effects, on the one hand, of a
part being largely developed through natural selection and another and adjoining part
being reduced by this same process or by disuse, and, on the other hand, the actual
withdrawal of nutriment from one part owing to the excess of growth in another and
adjoining part (Darwin 1859, p. 147)."

The potential for negative associations between body parts to arise during

developmental processes is a long-appreciated phenomenon. The partitioning of

finite resources among developing traits may create trade-offs with distinct

consequences for the evolution of both morphology and life-history strategies

(Trumbo 1999). As selection can only act on available variation, such trade-offs may

constrain evolution to the pathways allowed by developmental processes (Cheverud

1984; Maynard Smith et al. 1985; Arnold 1992). Trade-offs between, for example,

somatic growth and reproduction have been studied extensively in a variety of taxa

including mammals, birds, lizards, insects, and flowering plants (Clutton-Brock et al.

1982; Roff 1992; Stearns 1992; Sinervo and Svensson 1998; Zera and Harshman

2001). However, the term 'trade-off' has often been applied with little or no

understanding of whether the associated traits are functionally coupled. For example,

negative associations between traits may arise due to molecular signaling pathways or

genetic linkage (e.g. Leroi 2001). A recent review has stressed that the term 'trade-

off' should constitute a specific hypothesis regarding a negative functional interaction

between traits (Zera and Harshman 2001), and this usage is adopted here.

1

Recently, holometabolous (completely metamorphic) insects have emerged as a model system for the study of trade-offs because several features of their developmental program provide a theoretical framework for the functional relationships between traits. First, holometabolous insect metamorphosis is defined by phases with distinct developmental boundaries (larva, pupa, and adult), and during development feeding is restricted to the larval phase (Nijhout 1994; Truman and Riddiford 1999, 2002). Body parts develop from precursors called imaginal discs, which are small, invaginated pockets of cells within the larva (Nijhout 1994; Chapman 1998). However, imaginal disc proliferation occurs primarily after feeding has ceased, requiring the growth of body parts to occur in a system that is closed to the acquisition of additional resources (Nijhout 1994). Furthermore, the amount of time spent feeding may be limited if the length of the larval phase is associated with risk of predation or parasitism, or if the resource is transient. Although adults of many insects feed, the exoskeleton is fixed soon after eclosion, preventing additional growth. In organisms that grow continuously as adults, trade-offs may be obscured because developmental demands can be met by changes in resource acquisition (Van Noordwijk and deJong 1986; Houle 1991). The development of holometabolous insects largely circumvents this problem. Second, the imaginal discs are often partitioned into more than one body part, and allocation of limited resources into one part precludes their allocation to another, making trade-offs among body parts arising from the same disc particularly likely (Riska 1986; Nijhout and Wheeler 1996).

A third aspect of holometabolous insect development that allows for predictions about trade-offs is the hormonal control of development. The growth of

imaginal discs is regulated by juvenile hormone (JH), which has been implicated in both morphological and life-history trade-offs and may serve as a link between environmental inputs (e.g. resource acquisition) and the growth of body parts (Dingle and Winchell 1997; Nijhout and Emlen 1998; Zera et al. 1998a, 1998b; Emlen and Nijhout 1999; Cisper et al. 2000; Salmon et al. 2001; Tatar et al. 2001; Flatt and Kawecki 2004; Flatt et al. 2005). Briefly, there is a short "JH sensitive" period just after the cessation of larval feeding during which the imaginal discs undergo rapid proliferation (Nijhout 1994; Emlen and Allen 2004). The JH titer at this time is affected by feeding rate and diet quality (deWilde and Beetsma 1982; Ono 1982; Trumbo and Robinson 2004) and can influence the phenotype of emerging adults as well as the relative sizes of body parts (Emlen and Nijhout 1999; Emlen and Allen 2004). Juvenile hormone may thus serve to communicate information about nutritional status and overall size to developing tissues, ultimately regulating the scaling relationships of body parts and potential trade-offs between them.

Furthermore, the growth of exaggerated, secondary sexual traits may make trade-offs likely if they require a significant amount of the resources acquired during the larval phase. Sexual selection theory predicts that exaggerated traits are sensitive to condition, thereby serving as reliable signals of quality to potential mates (Zahavi 1975; Iwasa et al. 1991; Andersson 1994). The size of an exaggerated ornament is typically explained in terms of opposing selective forces resulting from the fitness benefits incurred via increased mating success and the costs imposed by natural selection (Andersson 1994). However, developmental processes may constrain the short-term evolution of certain phenotypes (Cheverud 1984; Maynard Smith et al.

1985; Arnold 1992). For example, when traits arise from a common precursor, genetic changes may be constrained by the developmental framework in which they are expressed (Wagner 1989). In addition, 'genic capture' (Rowe and Houle 1996) models predict that, when resources are limited, condition-dependent traits should be more likely to experience trade-offs than traits which are insensitive to condition (Tomkins et al. 2004). Holometabolous insects that bear exaggerated traits are thus ideal model systems in which to explore both the causes and consequences of trade-offs.

II. STUDY SYSTEM

Stalk-eyed flies (Diptera, Diopsidae) are emerging as a model system for the study of the evolution of an exaggerated secondary sexual trait (Burkhardt et al. 1994; Wilkinson and Taper 1999; David et al. 2000; Baker and Wilkinson 2001). All members of the family possess eyes that are laterally displaced away from the head on elongated scleritized "stalks." Relative eye-span exhibits dramatic variation within the family, and a phylogenetic analysis of 30 species reveals that sexual dimorphism for relative eye-span has undergone several independent gains and losses (Baker and Wilkinson 2001). Relative eye-span responds to artificial selection (Wilkinson 1993) and substantial additive genetic variance persists even when dimorphism is extreme (Wilkinson and Taper 1999), suggesting that eye-span exaggeration is not constrained by available variation. Trade-offs arising from developmental or physiological mechanisms are thus promising avenues for exploring costs of exaggerated eye-span.

In this study, I use two congeners that differ in relative eye-span: *Cyrtodiopsis dalmanni* (Fig. 1) exhibits sexual dimorphism (Wilkinson 1993), while *C. quinqueguttata* (Fig. 2) is sexually monomorphic (Baker and Wilkinson 2001). Male eye-span in *C. dalmanni* is sexually selected via female choice (Wilkinson and Reillo 1994) and intrasexual contest competition (Panhuis and Wilkinson 1999). In accordance with sexual selection theory, male eye-span in *C. dalmanni* also displays condition dependence (David et al. 1998, 2000; Cotton et al. 2004a), whereas eye-span in *C. quinqueguttata* appears to be unaffected by condition (Wilkinson and Taper 1999). *C. dalmanni* and *C. quinqueguttata* therefore allow comparisons of related species with dissimilar relative eye-span shaped by different selective environments. In addition, studies of the development and morphology of the eye-antennal disc, as well as emerging information about gene expression within these discs, has afforded a new perspective on the development of eye-stalks (Buschbeck and Hoy 1998; Buschbeck et al. 2001; Hurley et al. 2001, 2002). We therefore have both an evolutionary and developmental context within which the results of the present experiments can be evaluated.

III. EXPERIMENTAL APPROACHES

Historically, three main empirical approaches have been utilized in investigations of trade-offs: 1) measurement of unmanipulated phenotypes, 2) experimental manipulation of phenotypes, and 3) genetic analyses.

Each approach has unique benefits and drawbacks, and the relative merits of each have been debated (Sinervo and Basolo 1996; Zera et al. 1998a; Zera and

Harshman 2001). For example, correlations in unmanipulated organisms can suggest physiological causes of trade-offs but can only provide limited information about the underlying mechanisms (Zera and Harshman 2001). In addition, when nutrient intake is uncontrolled, variable resource acquisition may obscure trade-offs (Van Noordwijk and deJong 1986). This problem can be largely circumvented with experimental manipulations of the phenotype, for example by altering diet quality or quantity. This approach greatly increases the range of phenotypes to make detection of trade-offs more likely, but may also uncouple traits from the mechanisms that link them (Sinervo and Basolo 1996). Another drawback is that the levels of resources most likely to elicit a response are at the extremes, perhaps even beyond the ranges normally encountered in nature, thereby complicating the interpretation of the results (Cotton et al. 2004a). Hormonal manipulations can also be used to alter phenotypes ('phenotypic engineering') in a more controlled manner, and this approach benefits from its ability to highlight physiological mechanisms underlying trade-offs (Ketterson and Nolan 1992; Nijhout and Emlen 1998; Zera et al. 1998a; Emlen and Nijhout 1999; Emlen 2000). However, application of exogenous hormones can have pleiotropic effects on other traits that may ultimately alter the trade-off under investigation (Zera et al. 1998a). Finally, genetic approaches, most notably artificial selection experiments, have emerged as powerful tools for assessing the genetic basis of trade-offs (Fuller et al. 2005). Like methods that manipulate phenotypes, perturbation of the genotype may also alter trade-offs and the mechanisms that underlie them (Partridge and Sibly 1991). Ultimately, a complementary approach based on multiple methods is required for a thorough understanding of trade-offs and

6

their underlying mechanisms (Zera and Harshman 2001). I have therefore used a combination of approaches including artificial selection, hormone (JH) application, and diet manipulation to examine trade-offs in the present study.

IV. PRESENT STUDY

In Chapter 1, I use *C. dalmanni* artificially selected for long and short relative eye-span to ask whether selection on eye-span has resulted in correlated changes in other features of head morphology, including eye-stalk width and eye-bulb area. Bidirectional sexual selection was exerted on males for 60 generations in the laboratory, resulting in a significant divergence in relative eye-span in the high and low lines. I measured the morphology of adult males and females in generations 58, 59, and 60. The results of this experiment reveal strong negative relationships between eye-span and both eye-stalk width and eye-bulb area in males but not females. The implications of morphological trade-offs acting as constraints on eye-span are discussed.

In Chapter 2, I administer a JH agonist, methoprene, to developing *C. dalmanni* larvae. Measurements of adults indicate that JH changed the static allometry of eye-span in males but not females. Small-bodied males in high JH treatments were induced to produce longer eye-stalks than expected for their body size. In addition, adult males treated with JH as larvae exhibited compromised testis development and sperm production. Female gonad development was unaffected by larval hormone applications. The negative relationship between eye-span and testes indicates that a trade-off can occur across a developmental boundary. These results are consistent with JH-mediated resource allocation trade-offs between eye-span and

7

testes, and indicate that reduced testis size may be a potential cost for the production of an exaggerated trait.

In Chapter 3, I directly manipulate resources by rearing *C. dalmanni* and *C. quinqueguttata* in diets that vary in quality. Measurements of adult morphology indicate that eye-span in male *C. dalmanni* alone is sensitive to condition. Despite changes in relative eye-span with diet quality, no trade-offs were recorded between eye-span and eye-bulb area or eye-stalk width. Testis size, however, was significantly reduced in the lowest quality diet in male *C. dalmanni*, suggesting that environmental stress caused males to allocate resources to eye-span at the expense of testes. No trade-offs were observed in either female *C. dalmanni* or either sex of *C. quinqueguttata*. The trade-off between eye-span and testes in male *C. dalmanni* mimics the trade-off caused by JH applications, thereby supporting a hormone-mediated resource allocation hypothesis.

V. SIGNIFICANCE

The results presented here suggest that eye-span exaggeration in stalk-eyed flies may result in trade-offs both within and between primary and secondary sexual traits. In addition, these results are consistent with models of hormone-mediated trait scaling and differential resource allocation during development. Previous work on this topic has focused almost exclusively on insects with discontinuously scaled traits (i.e. polyphenisms: Emlen 1997, 2000, 2001; Emlen and Nijhout 1999; Nijhout 2003), and the present experiments provide the first evidence that the allometry of a continuously scaled trait, as well as trade-offs associated with that trait, may be

hormonally regulated. While this study focused on a holometabolous insect, the implications for JH-mediated resource allocation trade-offs need not be restricted to this group, as JH is also found in other arthropods (Tobe and Bendena 1999). In addition, the pleiotropic nature of hormones and hormone signaling suggests that endocrine regulation may provide a general mechanism underlying trade-offs in both invertebrates and vertebrates (Ketterson and Nolan 1992; Finch and Rose 1995; Dingle and Winchell 1997; Sinervo and Svensson 1998; Flatt et al. 2005). Here, artificial selection, hormone applications, and manipulation of diet quality elicited trade-offs between both near and distant body parts that persisted across a developmental boundary, emphasizing that trade-offs may not necessarily occur in spatial or temporal proximity.

FIG. 1. Male (right) and female (left) *Cyrtodiopsis dalmanni*. Photo: A. Lorsong.

FIG. 2. Male (left) and female (right) *Cyrtodiopsis quinqueguttata*. Photo: A. Lorsong.

1 mm
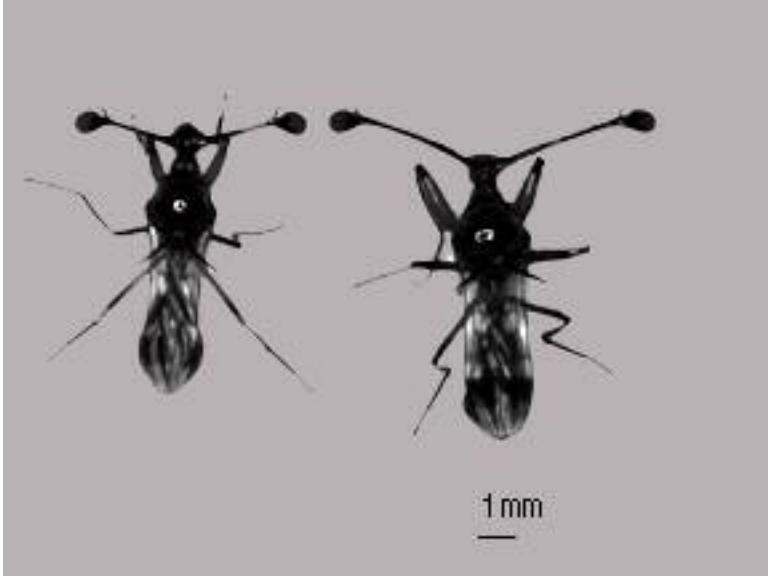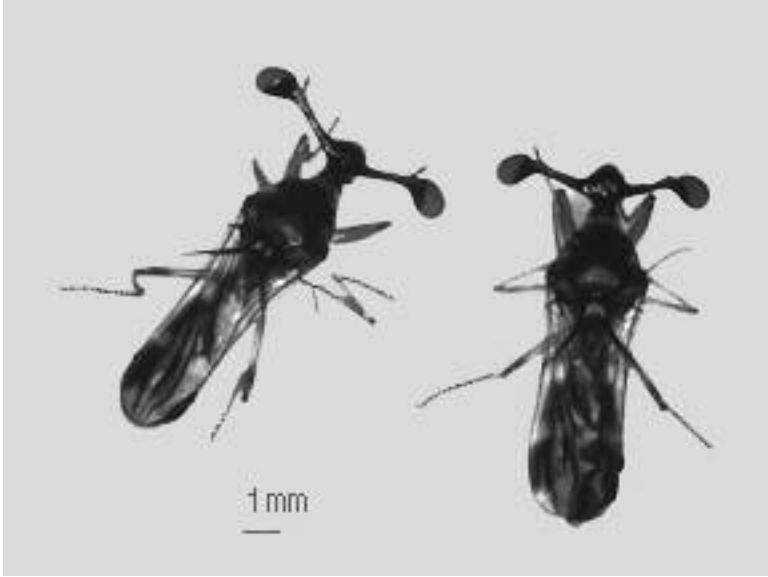
CHAPTER 1: CORRELATED RESPONSES TO ARTIFICIAL SELECTION ON MALE EYE-SPAN REVEAL MORPHOLOGICAL TRADE-OFFS IN STALK-EYED FLIES

ABSTRACT

While there is compelling evidence demonstrating the selective advantage of exaggerated secondary sexual traits, much less is known about the costs that may limit further elaboration of these traits. Trade-offs (negative correlations) between exaggerated traits and other body parts may impose such costs, thereby constraining their joint evolution. In particular, negative genetic correlations may arise when two traits are developmentally or physiologically coupled. Here, I use stalk-eyed flies (*Cyrtodiopsis dalmanni*) artificially selected for increased or decreased male relative eye-span to assess potential trade-offs between exaggerated eye-span and other morphological traits. As the head and eyes arise from a single developmental precursor, the eye-antennal imaginal disc, theory predicts that trade-offs between these structures are likely to occur during development. The results of this study support this prediction: male eye-span shows strong negative genetic correlations with both the width of the eye-stalk and the size of the eye itself. In contrast, wing length in males demonstrated only a weak trade-off with eye-span. In females, a weak trade-off was observed between eye-span and the size of the eye, but there were no significant relationships between eye-span and any other trait. These results suggest that trade-offs may limit additional eye-span elaboration and, when evaluated

with the results of previous studies, are consistent with resource allocation trade-offs

arising during development.

Allometry, or the scaling relationships of morphological traits with body size, has been of central interest to biologists for nearly a century (Thompson 1917; Huxley 1932; Cock 1966). Specific focus has been given to 'static' allometry, or the scaling relationship among individuals between one appendage or organ and body size (Huxley 1932; Cock 1966). The static allometry of exaggerated secondary sexual traits or 'ornaments' in particular has attracted attention because sexual selection theory makes specific predictions about the selection pressures shaping the final size of these traits (Petrie 1992; Simmons and Tomkins 1996; Knell et al. 1999; Baker and Wilkinson 2001; Cotton et al. 2004b). Specifically, the size of an exaggerated ornament is typically explained in terms of opposing selective forces resulting from the fitness benefits incurred via increased mating success and the costs imposed by natural selection (Andersson 1994). Artificial selection experiments as well as comparative studies across taxa offer clear evidence that the allometric scaling relationships of exaggerated traits are evolutionarily labile (reviewed in Emlen and Nijhout 2000). However, the underlying developmental mechanisms that regulate or generate such scaling relationships have only recently been explored in earnest (Nijhout and Wheeler 1996; Stern and Emlen 1999; Emlen and Nijhout 2000; Brakefield et al. 2003; Emlen and Allen 2004).

The translation of genotype to phenotype occurs via developmental and physiological pathways that operate during ontogeny, and holometabolous insects have recently emerged as a model system for the study of these pathways (Nijhout and Wheeler 1996; Stern and Emlen 1999; Emlen and Nijhout 2000; Emlen and Allen

2004). While trait exaggeration in insects can result in some strikingly unusual morphologies, the variety of traits or combinations thereof is not infinite, suggesting that some body plans are more easily created than others (Emlen 2000; Emlen and Nijhout 2000). The notion that developmental processes may somehow constrain the evolution of certain phenotypes is not new, and is certainly not restricted to insects (Cheverud 1984; Maynard Smith et al. 1985; Arnold 1992). However, the particular manner in which holometabolous insects develop may render constraints particularly relevant within this group. During development, resources are acquired during the larval phase, but the imaginal discs that give rise to adult structures undergo proliferation and differentiation chiefly after the cessation of feeding (Nijhout 1994). The exoskeleton is fixed soon after eclosion, thereby confining the growth of appendages to the late larval and pupal phases. This pattern of development results in traits that must compete within a closed system for a finite pool of resources, which may in turn influence their allometries (Nijhout and Wheeler 1996; Stern and Emlen 1999). For example, the growth of exaggerated mandibles in stag beetles (Knell et al. 2004) and 'antlers' in tephritid flies (Wilkinson and Dodson 1997) demonstrates a curved allometry for larger individuals, suggesting that the depletion of resources may limit further elaboration.

Theoretical models of imaginal disc growth also predict that changes in rates or amounts of proliferation in one disc will result in compensatory responses in other discs (Nijhout and Wheeler 1996). If discs compete for a limiting resource during development, it follows that trade-offs between traits may arise as a consequence of this competition. The most compelling support for this prediction comes from the

16

well-studied horned beetles (*Onthophagus*), where trade-offs have been recorded between the exaggerated horns and eyes (Nijhout and Emlen 1998; Emlen 2001), antennae (Emlen 2001), wings (Emlen 2001; Tomkins et al. 2005), and genitals (Moczek and Nijhout 2004). Studies of stag and rhinoceros beetles also reveal trade-offs between exaggerated mandibles and wings (Kawano 1997) or horns and wings (Kawano 1995), respectively. Additional evidence for competition between body parts can be found in *Precis* butterflies, where experimental removal of hindwing imaginal discs causes compensatory growth of legs and forewings (Klingenberg and Nijhout 1998; Nijhout and Emlen 1998).

Trade-offs such as those described in butterflies and beetles may be borne out as genetic correlations between phenotypic traits. Response to selection on a given trait depends both on the heritability of the trait under selection and the genetic and phenotypic covariances among traits (Falconer 1960). Genetic correlations between morphological traits have historically been predicted to be more positive than correlations between other traits (Roff 1997). For example, studies of genetic correlations in *Drosophila* frequently report positive correlations among most traits measured (Cowley and Atchley 1990; Wilkinson et al. 1990; Norry et al. 1997). However, negative correlations may be expected when the traits are developmentally linked (Riska 1986), thereby imposing a constraint upon their joint evolution (Cheverud 1984; Arnold 1992). Artificial selection experiments are a powerful tool for revealing genetic or phenotypic correlations consistent with constraints on evolution (Fuller et al. 2005). For example, artificial selection for increased horn length in *Onthophagus* beetles resulted in smaller eyes, suggesting that reduced visual

acuity constrains horn evolution (Nijhout and Emlen 1998; Emlen 2000). However, apart from a handful of studies, evidence suggesting that genetic correlations underlie morphological trade-offs is surprisingly rare (Trumbo 1999).

Here, I test the prediction that artificial selection for increased trait exaggeration results in trade-offs in stalk-eyed flies (Diptera, Diopsidae). *Cyrtodiopsis dalmanni* have exaggerated eye-span resulting from a lateral displacement of the eyes on elongated scleritized "stalks." *C. dalmanni* also exhibit sexual dimorphism such that male eye-span is greater than their body length and female eye-span is less than their body length (Wilkinson 1993; Baker and Wilkinson 2001). Male eye-span is a classic example of a sexually selected character under selection by both choosy females (Wilkinson and Reillo 1994) and intrasexual contest competition (Panhuis and Wilkinson 1999). Artificial selection experiments demonstrate that bidirectional selection on relative eye-span in male *C. dalmanni* results in significant divergent responses in high and low lines within 10 generations (Wilkinson 1993). Selection has now been maintained in the laboratory for 60 generations, and although relative eye-span has reached a plateau in both high and low lines, significant differences between lines persist after 57 generations (Wilkinson et al. 2005). In this study, I test the hypothesis that eye-span exaggeration results in negative genetic correlations with other traits by measuring adult flies from these selected lines. As both the eyes and eye-stalks originate from the eye-antennal imaginal disc (Buschbeck et al. 2001; Hurley et al. 2002), evidence of negative associations between structures arising from this disc would support the prediction that developmentally coupled traits should trade off (Riska 1986). Therefore, I first

test for within-disc trade-offs between eye-span, eye-stalk width, and eye-bulb area. I then test for a trade-off between eye-span and wing length, representing a between-disc comparison. The results of these experiments suggest that trade-offs may act as a constraint limiting eye-span exaggeration.

MATERIALS AND METHODS

Fly stocks

C. dalmanni flies used in this study were descended from individuals captured near Kuala Lumpur, peninsular Malaysia in January 1989. In the laboratory, adults were housed in a 40 x 40 x 120 cm plexiglass population cage maintained in a 25º C constant temperature room on a 12L:12D cycle with at least 75% relative humidity. Adults feed and oviposit on a mixture of pureed corn containing 1% methylparaben as a mold inhibitor (Wilkinson 1993). Eggs were collected by introducing plastic cups containing approximately 50 mL of corn puree to the cage twice weekly. These cups were then incubated at the conditions described above and emerging adults were returned to the population cage as necessary to maintain approximately 300 individuals. Prior to selection, *C. dalmanni* were reared in the laboratory for approximately seven generations (Wilkinson 1993).

Selection regime

Beginning in March 1991, artificial sexual selection was exerted on the ratio of eye-span to body length in male flies (Wilkinson 1993). Selection was imposed by housing 10 sexually mature males having either the highest or lowest ratios of 50

19

measured males with 25 randomly chosen virgin females. Four replicate lines were

created in this manner, with two each of the "high" and "low" selected lines. Two

unselected control lines were created at the same time by randomly selecting 25

females and 10 males from the population. Adults of each replicate were maintained

in 40 x 40 x 40 cm cages incubated as described above. To collect offspring for the

next generation, selected adults were first allowed to breed for two weeks. Plastic

cups containing approximately 50 mL of pureed corn were then introduced into the

cages twice weekly. Newly eclosed flies were maintained as virgins in single-sex

cages for at least four weeks until selection. This selection regime was followed until

generation 31, when selection was relaxed by choosing 25 of 50 measured males in

order to reduce inbreeding (Wilkinson et al. 2005).

Morphological measurements

In this study, I measured flies from each of the six lines after 58, 59, and 60

generations of selection. After eclosion, I obtained morphological measurements of

each individual by placing live flies under CO_2 anesthesia and viewing a video image

digitized into a computer. Each fly was placed in an identical position, resting on the

thoracic and interorbital spines. Using the program NIH Image (ver. 1.59, National

Institutes of Health, Bethesda, MD), I measured eye-span between the outer edges of

the ommatidia, body length from head to wing tip, and wing length from the point of

insertion to the wing tip. Only one wing was measured on each individual as

previous studies have demonstrated that wing length asymmetry is unaffected by

larval condition (David et al. 1998; Cotton et al. 2004a). I also measured the width of

the eye-stalk immediately adjacent to the interorbital spine and the area of the

pigmented optical surface of the eye bulb. All measurements were taken to the

nearest 0.01 mm at a resolution of 50 pixels/mm. Eye-span, body length, eye-bulb

area, and eye-stalk width measurements include 101 low, 89 control, and 116 high

females, and 80 low, 111 control, and 114 high males. Wing length was measured for

a subset of these individuals, including 75 low, 76 control, and 80 high females, and

65 low, 75 control, and 70 high males.

Statistical analyses

All analyses were performed using JMP version 5.0 (SAS Institute, Cary, NC,

USA). All pairwise comparisons are post-hoc Fisher's PLSD tests and errors are

indicated by ± SE. Non-significant interactions were sequentially removed from the

models unless otherwise indicated, and sequential Bonferroni corrections for multiple

tests were used where needed.

RESULTS

A mixed model nested ANCOVA was used to determine if body length,

generation, selected line, or replicate influenced each measured trait in males and

females. I nested replicate within selected line, assumed that replicate was a random

effect, and included body length as the covariate. As each trait differed significantly

between the sexes ($F_{1, 603} = 31.14 – 9431.17$, all $P < 0.0001$), I tested males and

females separately. I detected a significant generation effect for eye-span in both

sexes (females: $F_{2, 289} = 28.33$, $P < 0.0001$; males: $F_{2, 288} = 55.12$, $P < 0.0001$),

suggesting that either environmental variation or the selection regime resulted in a

change in eye-span in the three generations measured. Pairwise comparisons revealed that generations 58 and 59 did not differ in overall body length ($P = 0.24$), but did indicate a significant decline in generation 60, where body length was smaller than in the preceding two generations (both comparisons $P < 0.0001$). This effect was present in both sexes and all three lines, suggesting that the generation effect is most likely attributable to variation in the larval environment, and is not a directional effect of the selection regime. Individuals from each generation were therefore combined for subsequent analyses.

Differences in relative eye-span between selected lines were caused by changes in both absolute eye-span and body length, although the magnitude of the effect was greater for absolute eye-span. In both sexes, absolute eye-span differed by selected line (females: $F_{2,300} = 182.67$; males: $F_{2,229} = 403.52$, both $P < 0.0001$) and replicate (females: $F_{2,300} = 17.46$; males: $F_{2,229} = 35.09$, both $P < 0.0001$). Similarly, body length demonstrated significant effects of line (females: $F_{2,300} = 15.81$; males: $F_{2,229} = 35.59$, both $P < 0.0001$) and replicate (females: $F_{2,300} = 3.36$, $P = 0.01$; males: $F_{2,229} = 16.29$, $P < 0.0001$). After controlling for body size effects, nested ANCOVAs explained significant variation in every trait measured (Table 1). In males, significant effects of selected line were detected for every trait, and significant effects of replicate for all traits except wing length. In females, significant effects of replicate were detected for all traits, and of line for all traits except wing length (Table 1; Fig. 3).

In order to determine if the changes in eye-span in the selected lines were associated with changes in other traits independent of body size, I calculated the

residuals of the least squares regression of each trait on body size. Since the differences in body size between generations increases variation that could obscure trade-offs, I calculated residuals separately by sex within each generation and pooled the results for subsequent analyses. I first tested both sexes together using a mixed model nested ANCOVA as described above, with residual eye-span as the covariate instead of body size. The full model revealed significant interactions between sex and residual eye-span for each trait except wing length (residual eye-bulb area: $F_{1,598} = 16.56$, $P < 0.0001$; residual eye-stalk width: $F_{1,598} = 13.59$, $P < 0.0001$; residual wing length: $F_{1,428} = 2.15$, $P = 0.14$), suggesting that male and female head morphology did not respond similarly to selection on male eye-span. The sexes were therefore separated for subsequent analysis. Trade-offs are expected to result in significant negative slopes of the least squares regression of each residual trait on residual eye-span. In males, significant negative slopes were detected for each trait, while in females only eye-bulb area demonstrated a significant negative relationship with eye-span (Table 2; Fig. 4). Residual eye-stalk width and wing length in females had no significant associations with residual eye-span (Fig. 4).

DISCUSSION

Artificial sexual selection resulted in a strong bidirectional response in male relative eye-span that altered eye-span allometry, as documented previously (Wilkinson 1993; Wilkinson et al. 2005). In male flies, increased eye-span exaggeration also resulted in significant negative associations with two other measures of head morphology: eye-bulb area and eye-stalk width (Fig. 4a, c). Wing

length also demonstrated a significant negative relationship with male eye-span, although this response was the weakest of the measured traits (Fig. 4e). The correlated response in female relative eye-span (Fig. 3a) has also been noted in previous studies (Wilkinson 1993; Wolfenbarger and Wilkinson 2001; Wilkinson et al. 2005) and suggests that genes influencing male eye-span also influence female eye-span. Eye-span in females demonstrated a significant negative association with eye-bulb area (Fig. 4b), but this relationship is primarily attributable to the increase in eye-bulb size in low lines rather than a decrease in high lines (Fig. 3b). No significant relationship between eye-span and either eye-stalk width or wing length was detected in female flies (Fig. 4d, f). Significant differences between the sexes were also noted for every trait: males have longer eye-span but smaller eye-bulbs and thinner eye-stalks than females, while females have shorter wings than males. Sex-specific differences in trait sizes indicate that selection on eye-span in males caused correlated changes in head morphology.

Collectively, these results suggest that selection for increased eye-span exaggeration in male stalk-eyed flies results in trade-offs with the size of the eye and the thickness of the eye-stalk. Negative genetic correlations between phenotypic traits, often arising via pleiotropy or linkage, may influence the direction of morphological evolution and therefore the range of possible forms (Falconer 1960; Roff 1997). Furthermore, negative correlations are frequently interpreted as a constraint on the further elaboration of a phenotypic trait (Cheverud 1984; Arnold 1992). In diopsid species, substantial additive genetic variance persists even when eye-span dimorphism is extreme (Wilkinson and Taper 1999). In addition, response

to selection in *C. dalmanni* is symmetric in high and low lines (Wilkinson 1993).

Together, these studies suggest that an increase or decrease in relative eye-span may

be equally likely. However, the results presented here suggest that reductions in eye-

bulb size and eye-stalk width may pose a constraint on increased eye-span

elaboration. While direct costs associated with these morphological changes are

currently unknown, even the moderate eye-span exaggeration in the sexually

monomorphic species *C. quinqueguttata* may compromise visual processing

(Buschbeck and Hoy 1998). This effect would only be exacerbated if reductions in

eye-bulb size also reduce the number or size of ommatidia. In addition, eye-span

exaggeration in *C. whitei*, a species morphologically similar to *C. dalmanni*, is

associated with reduced flight performance consistent with either aerodynamic or

visual processing effects (Swallow et al. 2000). It seems reasonable to assume,

therefore, that exaggerated eye-span could be costly.

While genetic correlations may reveal suggestive relationships among

phenotypic traits, limited information can be inferred unless it is known to what

extent the traits are functionally coupled. One review suggests that correlations

within a population are most useful as an expression of physiological or

developmental trade-offs within an individual (Stearns et al. 1991). Several aspects

of stalk-eyed fly development provide a theoretical framework for the trade-offs

reported here. First, the eye-stalk and eye both arise from a common precursor, the

eye-antennal imaginal disc (Buschbeck et al. 2001; Hurley et al. 2002). Models of

morphological correlations predict that traits arising from the same developmental

precursor are more likely to demonstrate trade-offs (Riska 1986). In *Drosophila*,

traits arising from the same disc have higher genetic correlations than traits from different discs (Cowley and Atchley 1990). A similar pattern may occur in stalk-eyed flies given that wing length displayed only a weak trade-off with eye-span. However, several alternative explanations may account for this relationship, including the smaller sample size for wing measurements, greater variation, and the possibility that wing length is also under selection to maintain flight (Swallow et al. 2000).

A second connection between eye-span trade-offs and development is suggested by comparisons with *Drosophila*. While many genetic correlations between body parts in *Drosophila* are positive (Cowley and Atchley 1990; Norry et al. 1997), an exception appears to exist for the eyes and head. In cactophilic *Drosophila*, there is a significant negative correlation between eye width and face width, structures that both arise from the eye-antennal imaginal disc (Norry et al. 2000). Interestingly, the measurements of face width and eye width described in this study are nearly analogous to the eye-span and eye-bulb area measurements used here. In addition, experiments with *D. melanogaster* indicate that two genes regulating head development, *wingless* (*wg*) and *decapentaplegic* (*dpp*), have antagonistic signaling pathways (Bessa and Casares 2001). The fate of cells from the same initial pool must therefore be assigned to either the eye (promoted by *dpp*) or head (promoted by *wg*) during development (Bessa and Casares 2001). While stalk-eyed flies have markedly different head morphology relative to *Drosophila*, the expression of three regulatory genes, including *wg*, is remarkably similar in the eye-antennal discs of both species (Hurley et al. 2001). The observed trade-offs in stalk-

eyed flies may therefore be explained in part by interactions between these regulatory genes or their signaling pathways.

Third, like all holometabolous insects, the proliferation and differentiation of the eye-antennal imaginal disc occurs after feeding has ceased. While additional feeding to acquire more resources may provide an apparent solution to resource limitation, opposing selection to avoid exposure to parasites may limit the length of the larval phase (Wilkinson 1993). If the eye-stalk and eye compete within a closed system for a shared limiting resource, theory predicts that an increase in one trait should result in a compensatory decrease in the other (Nijhout and Wheeler 1996; Stern and Emlen 1999). Recently, several authors have suggested that morphological trade-offs result from inter- or intra-disc competition for a hemolymph-borne growth factor or hormone (Klingenberg and Nijhout 1998; Moczek and Nijhout 2004; Tomkins et al. 2005) that responds either directly or indirectly to nutrition (reviewed in Emlen and Allen 2004). Differences in rates or amounts of imaginal disc proliferation may in turn influence the absolute and relative sizes of adult traits, providing a link between environmental inputs and allometry (Stern and Emlen 1999; Truman and Riddiford 2002; Emlen and Allen 2004).

Recent reviews have stressed that genetic changes altering responses to resources could ultimately act as a general mechanism underlying trait allometries (Stern and Emlen 1999; Emlen and Allen 2004). Indeed, resource allocation trade-offs are included as an assumption in a recent theoretical model of the evolution of allometry in sexually selected ornaments (Bonduriansky and Day 2003). Theory also predicts that, for pairs of resource-limited traits, variation in resource allocation can

lead to negative genetic correlations (Houle 1991). Empirical support for these ideas is provided by a study of *Onthophagus* beetles, where artificial selection on relative horn length resulted in correlated changes in the size of the eye (Nijhout and Emlen 1998). Negative genetic correlations between horns and eyes prompted the authors to suggest that resource allocation trade-offs may act as a developmental source of genetic constraint on horn evolution (Nijhout and Emlen 1998). The negative genetic correlations reported here may thus be a reflection of resource allocation trade-offs arising from competition for a shared, finite resource.

While the present study cannot determine the precise genetic or developmental mechanism underlying trade-offs, other studies of *C. dalmanni* support the involvement of resources in determining eye-span. For example, experiments manipulating condition via larval density (David et al. 1998) and food quantity (Cotton et al. 2004a) have shown that male eye-span is more condition-dependent than either female eye-span or other non-sexual traits. In addition, genetic variation underlies the response of male eye-span to environmental stress (David et al. 2000). Male eye-span is thus sensitive to resources, and genetic changes may mediate this sensitivity. Manipulation of larval diet quality has also revealed that males in the most stressed treatment demonstrate trade-offs between eye-span and testis size (Chapter 3). Juvenile hormone, a key regulator of development and metamorphosis, induced a similar trade-off in males (Fry 2006). While another study of the selected lines did not reveal any changes in testis size in high or low lines, a different method for measuring testes was employed than in the preceding two studies (Wilkinson et al. 2005). In addition, only males who successfully mated contribute to

the next generation of selected line flies, leading to the possibility that the selection regime also selected on testis size, thereby obscuring any trade-offs. While the nature of the trade-offs in these studies differs from that presented here, these results nevertheless collectively indicate that nutrition, hormones, and genetic changes, or interactions between them, may all contribute to trade-offs in stalk-eyed flies. Additional experiments will be required to disentangle environmental, physiological, and genetic effects; however, these results are consistent with recent models of trait scaling in insects (Stern and Emlen 1999; Emlen and Allen 2004).

In summary, artificial sexual selection on male relative eye-span in *C. dalmanni* results in trade-offs that may contribute to costs of eye-span elaboration. Evidence demonstrating the selective advantage of exaggerated secondary sexual traits has been well-documented (Andersson 1994), but there is less support for costs that are expected to oppose these benefits. The results presented here add to the relatively small number of studies suggesting that exaggerated traits in insects may be constrained by trade-offs, possibly as a result of the depletion of resources within the pupa (Nijhout and Emlen 1998; Emlen 2001; Knell et al. 2004). Trade-offs between eye-span and other morphological traits may explain why, despite sufficient additive genetic variation for eye-span (Wilkinson 1993; Wilkinson and Taper 1999), the degree of elaboration in flies selected for increased eye-span is rarely observed within the Diopsidae (Baker and Wilkinson 2001). However, male *C. dalmanni* from a population in Brastagi, Sumatra, have eye-span more than three times their body length (Swallow et al. 2005), suggesting that the constraints proposed here are not necessarily insurmountable. Further experiments are warranted to determine to what

extent natural selection may be able to overcome the trade-offs observed in the selected lines.

TABLE 1. F-ratios from nested ANCOVAs on eye-span, eye-bulb area, eye-stalk width, and wing length with body length as a covariate. Significant *P*-values are indicated at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.0001$ (***).

Source of variation	df	eye-span	eye-bulb	eye-stalk	wing
Males					
Body length	1	467.3***	65.1***	1.2	406.4***
Selected line	2	574.4***	172.9***	199.3***	9.0**
Replicate (line)	3	25.6***	4.0**	2.8*	2.3
Error	298				
Females					
Body length	1	429.4***	135.2***	40.2***	567.7***
Selected line	2	240.8***	39.6***	25.4***	2.4
Replicate (line)	3	19.2***	2.8*	18.3***	3.2*
Error	299				

TABLE 2. The results of least squares regressions of each residual trait on residual eye-span.

	n	β ± SE	t	*P*
Males				
Eye-bulb area	305	- 0.09 ± 0.006	15.63	< 0.0001
Eye-stalk width	305	- 0.02 ± 0.001	17.71	< 0.0001
Wing length	210	- 0.04 ± 0.013	3.06	0.002
Females				
Eye-bulb area	306	- 0.03 ± 0.012	2.27	0.02
Eye-stalk width	306	- 0.004 ± 0.002	1.68	0.10
Wing length	231	0.026 ± 0.025	1.08	0.28

FIG. 3. Least squares means of morphological traits after bidirectional selection on male relative eye-span: a) eye-span (mm), b) eye-bulb area (mm^2), c) eye-stalk width (mm), and d) wing length (mm). Error bars indicate ± SE and bar colors indicate replicates 1 (gray) and 2 (white). L = low, C = control, H = high.

FIG. 4. Scatterplots and least squares regression lines for the residuals of each morphological trait as a function of residual eye-span. Symbols indicate selected line: low = open circles, control = filled squares, high = open triangles.
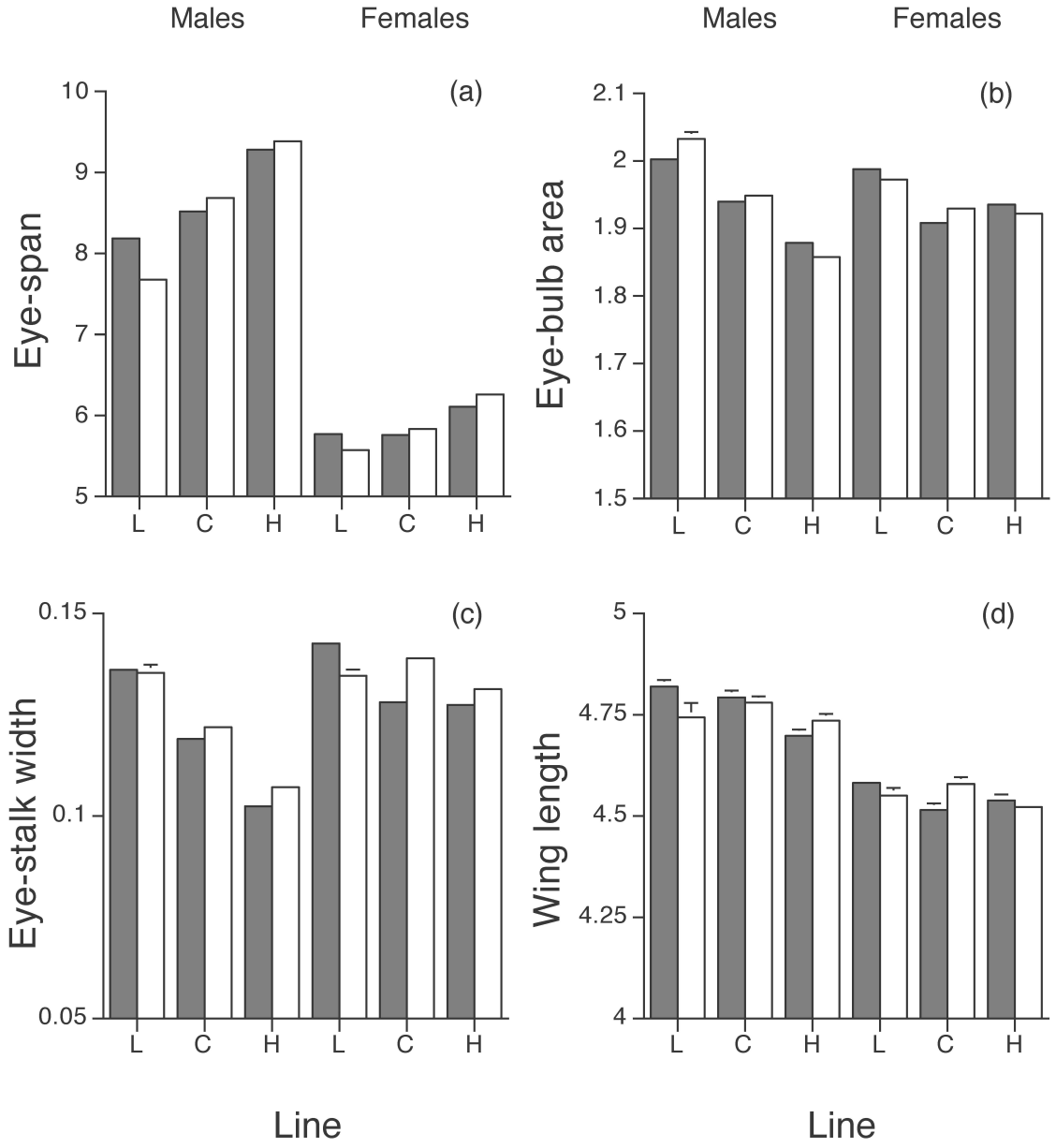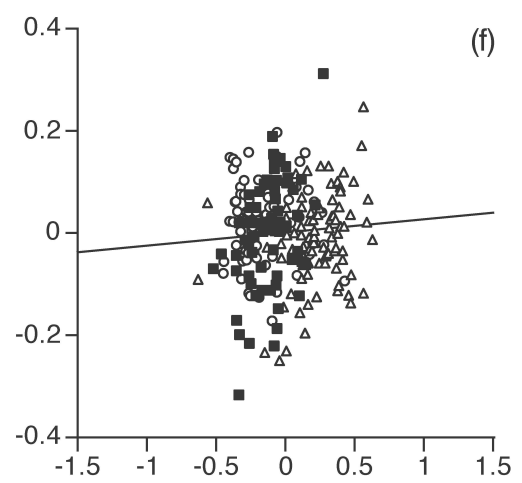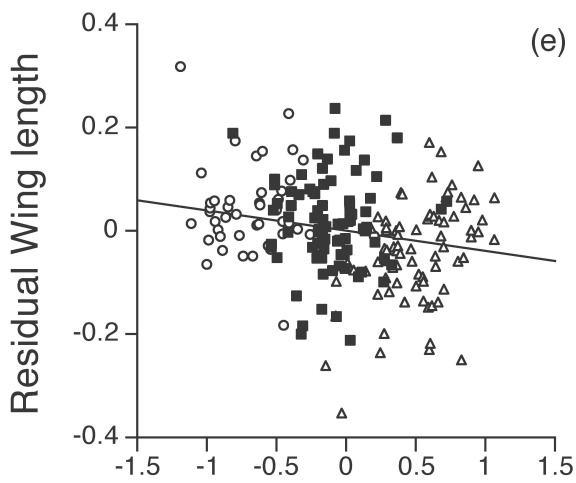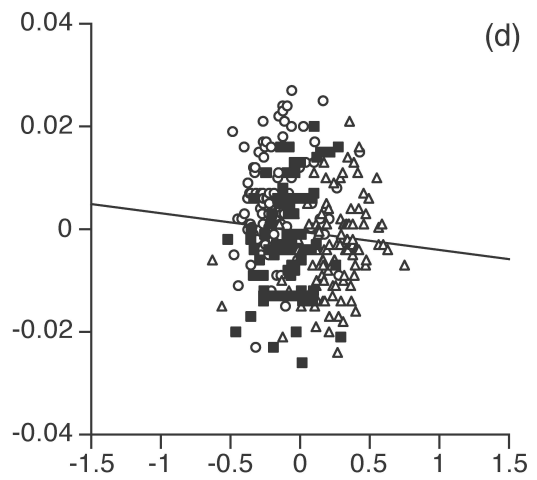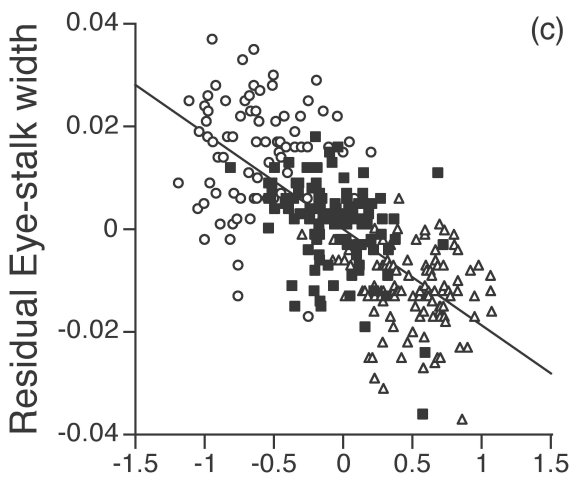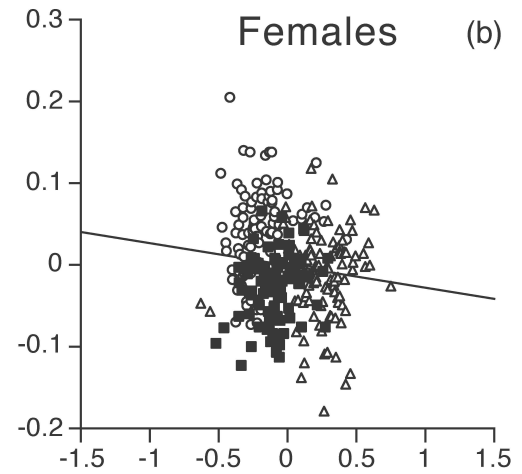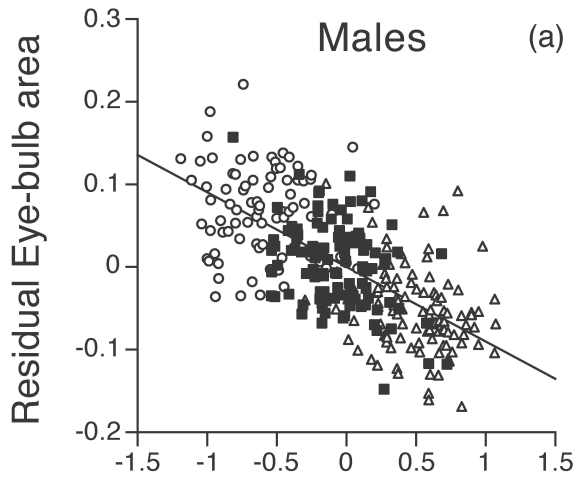
CHAPTER 2: JUVENILE HORMONE MEDIATES A TRADE-OFF BETWEEN PRIMARY AND SECONDARY SEXUAL TRAITS IN STALK-EYED FLIES

ABSTRACT

Trade-offs between developing body parts may contribute to variation in allometric scaling relationships in a variety of taxa. Experimental evidence indicates that both circulating levels of juvenile hormone (JH) and sensitivities of developing body parts to JH can influence morphology in polyphenic insects. However, the extent to which JH may regulate both the development of traits that scale continuously with body size and trade-offs between these traits is largely unknown. Here, I present evidence that the JH agonist methoprene applied to final instar larvae of a stalk-eyed fly (*Cyrtodiopsis dalmanni*) can induce males to produce larger eye-stalks relative to their body size. Examination of testis growth, sperm transfer and egg maturation indicates that JH induces a trade-off between eye-span and gonad development in adult males, but not females. Age at sexual maturity was unaffected by larval JH applications to either sex. Collectively, these results are consistent with JH-mediated allocation of resources to eye-span at the expense of testes, and indicate potential costs for the production of an exaggerated trait.

INTRODUCTION

Sexual selection theory presumes that secondary sexual traits impose some cost to the bearer that balances their fitness benefit. Exaggerated secondary sexual traits in particular have been predicted to incur energetic or physiological costs during adulthood that may limit further elaboration (Andersson 1994). However, trade-offs between exaggerated traits and other body parts during development may also have consequences for the evolution of morphology. Holometabolous insects provide an ideal system for examining trade-offs since adult structures grow from imaginal discs that proliferate during the pupal and late larval phases when the system is closed to the acquisition of new resources (Nijhout 1994). Theory therefore predicts that increased proliferation in one set of imaginal discs should result in compensatory changes in other discs (Nijhout and Wheeler 1996). Indeed, morphological trade-offs have recently been documented in butterflies (Nijhout and Emlen 1998), caddis flies (Stevens et al. 1999), and beetles (Kawano 1995; Emlen 2001).

While the nature and magnitude of trade-offs between body parts have been experimentally demonstrated in some species, the mechanism underlying these trade-offs is less clear. In order for the adult appendages to scale with body size, the larva must be able to assess its own body size and communicate that information to the growing tissues. Recent studies have suggested that juvenile hormone (JH) could be a messenger of such size information (Stern and Emlen 1999). This hormone has been known as a key regulator of insect development and metamorphosis for more than sixty years (Nijhout 1994) and has recently been implicated in both morphological and life-history trade-offs (Dingle and Winchell 1997; Nijhout and

37

Emlen 1998; Zera et al. 1998a). During development, there is a brief "JH sensitive"

period after the cessation of larval feeding during which the imaginal discs undergo

rapid proliferation (Nijhout 1994; Emlen and Allen 2004). The JH titer at this time

influences the phenotype of emerging adults (Emlen and Allen 2004) and, in bees and

ants, also regulates the size-dependent developmental shift between castes (deWilde

1976; Wheeler 1991). In horned beetles (*Onthophagus taurus*), applications of

exogenous JH to developing final-instar larvae can induce a switch from a hornless

phenotype to a horned one (Emlen and Nijhout 1999). In addition, *Onthophagus*

beetles demonstrate location-dependent trade-offs between horns and eyes, wings, or

antennae, depending on the position of the horns (Nijhout and Emlen 1998; Emlen

2001). Local resource utilization during development may therefore create

competition between neighboring body parts.

More recently, several studies have suggested that imaginal discs act as a sink

for a growth-regulating hormone like JH such that competition between body parts

may be mediated by the availability of JH to proliferating tissues, regardless of the

location of the competing traits (Moczek and Nijhout 2004; Tomkins et al. 2005).

Alternatively, the relative sensitivity of different discs to circulating hormones may

affect the onset or duration of cell proliferation and differentiation (Stern and Emlen

1999; Emlen and Allen 2004). In *O. taurus*, experimental ablation of the genital discs

that give rise to male genitalia resulted in disproportionately large horns (Moczek and

Nijhout 2004). A trade-off between horns and genitalia indicates that competition

during development is not necessarily limited to neighboring parts, and a separate

study of *O. taurus* specimens found additional evidence for trade-offs between horns

and wings, traits that do not grow in close proximity (Tomkins et al. 2005). Few studies have explicitly tested the involvement of JH in morphological trade-offs, but a recent review suggests that genetically controlled sensitivities to circulating hormones may ultimately determine the relative sizes of physical traits (Emlen and Allen 2004).

Nearly all of the exaggerated morphologies, including beetle horns, that have been examined for evidence of trade-offs are polyphenisms that show discontinuous or sigmoid scaling relationships relative to body size (Emlen and Nijhout 2000; Nijhout 2003). The alternative phenotypes of polyphenic insects are regulated by developmental switches that ultimately rely on threshold hormone levels to determine the adult phenotype (Zera et al. 1998a; Cisper et al. 2000; Nijhout 2003). While the developmental regulation of polyphenic traits has received much recent attention, it is still unknown to what extent JH may regulate the smaller-scale changes in phenotypic traits that scale continuously with body size. This is of particular relevance since polyphenisms are likely to have evolved from continuously scaled traits (Nijhout 2003).

Here, I use a holometabolous insect, the stalk-eyed fly (*Cyrtodiopsis dalmanni*), to examine whether JH administered during development may mediate a trade-off between two distant body parts, eye-stalks and gonads. Stalk-eyed flies of both sexes have eyes borne at the end of long scleritized cephalic projections, or "stalks." However, *C. dalmanni* exhibits sexual dimorphism such that male eye-span is greater than their body length and female eye-span is less than their body length (Wilkinson 1993). The exaggerated eye-span of males is strongly preferred by choosy females (Wilkinson and Reillo 1994), and is also correlated with the outcome

of male contest competition (Panhuis and Wilkinson 1999). Not surprisingly, male eye-span also displays condition dependence and is therefore tightly correlated with body size (David et al. 1998, 2000; Cotton et al. 2004a). Since eye-span demonstrates a continuous scaling relationship with body size (Wilkinson 1993), the expression of eye-stalks may not necessarily rely on a threshold level of JH. The final size of eye-stalks may, however, be regulated by variations in sensitivity to JH. I test this prediction by topically applying the JH agonist methoprene to developing larvae and measuring adult morphology. Evidence supporting JH-mediated development of eye-stalks in *C. dalmanni* will help evaluate how generalizable the hormonal regulation of exaggerated traits might be. In addition, I use individuals treated with methoprene during development to assess potential trade-offs between eye-span and adult gonad development, sperm transfer, and age at sexual maturity. Collectively, the results of these experiments suggest previously underappreciated costs of bearing an exaggerated trait.

MATERIALS AND METHODS

Fly stocks

Flies used in this study were descended from individuals captured near Ulu Gombak, peninsular Malaysia (3º 54´ N, 101º 26´ E) in August 1999. I housed 20 males and 20 females in a Nalgene cage (45 x 24 x 20 cm) lined with moist cotton and blotting paper. All flies were maintained on a 12 L:12 D cycle at 25º C with at least 75% relative humidity. Adults feed and oviposit on pureed corn treated with 10% methylparaben as a mold inhibitor (Wilkinson 1993). Plastic cups containing

approximately 50 mL of pureed corn were changed twice weekly and incubated at the conditions described above. Emerging adults were returned to cages as necessary to maintain 20 individuals of each sex.

Juvenile hormone treatments

In Dipteran insects, the last larval instar is characterized by a period of activity called the "wandering phase" (Nijhout 1994). During this phase, the larvae purge the contents of their guts and leave their food source in search of a pupation site. The wandering phase also contains a JH-sensitive period during which the hemolymph titer of JH is elevated (Nijhout 1994). In order to artificially augment the peak of JH activity, I removed wandering last instar larvae from breeding cups and treated them with the JH agonist methoprene (99% mix of isomers; Supelco #PS-1040). Methoprene is used in lieu of natural JH due to its greater stability in the hemolymph, increased duration of action, and highly repeatable ability to mimic the physiological actions of JH (Nijhout 1994; Riddiford 1994; Wilson 2004). Since hormone manipulations had not been previously attempted in this species, I first conducted a pilot study to determine effective dosages. I topically applied 1 μl of varying concentrations of methoprene dissolved in acetone (0.926 μg, 0.0926 μg, 0.00926 μg, and 0.000926 μg methoprene per larva) to the anterior dorsal surface of each larva using a Hamilton syringe (#80100). I also created an untreated control and an acetone control (1 μl acetone/larva). Fifty wandering phase larvae collected from the breeding cups described above were randomly assigned to each treatment. Immediately following the applications, larvae were placed in groups of 10 in 500 mL

containers lined with moist cotton and incubated at 25º C on a 12 L:12 D cycle. All

treated larvae pupated within 24 hours. Since JH is known to affect eclosion success

(Nijhout 1994), I examined differences in eclosion rates for each treatment. The

percentage of successfully eclosing individuals within each treatment group were:

0.926 μg methoprene/larva: 0%; 0.0926 μg methoprene/larva: 40%; 0.00926 μg

methoprene/larva: 64%; 0.000926 μg methoprene/larva: 80%; acetone control: 84%;

untreated control: 90%. I discontinued the highest and lowest methoprene treatments

since the highest was 100% lethal and pairwise comparisons (Fisher's PLSD with a

Bonferroni correction) indicated that the lowest was not different from the acetone

control ($P = 0.39$), the untreated control ($P = 0.07$), or the next highest dosage ($P =$

0.05).

After determining which dosages of methoprene were most likely to have an

effect on stalk-eyed fly development, I treated additional wandering phase larvae as

described above using both controls and the 0.0926 μg methoprene/larva (0.298 mM)

and 0.00926 μg methoprene/larva (0.0298 mM) dosages (hereafter referred to as

"high" and "medium", respectively). Eclosing adults included 80 untreated control,

272 acetone control, 239 medium JH, and 217 high JH flies. In order to examine

effects of methoprene on development time, a subset of these individuals was

monitored daily to record the time that elapsed between pupation and eclosion

(untreated control, $n = 63$; acetone control, $n = 114$; medium JH, $n = 77$; high JH, $n =$

58).

Morphological measurements

I obtained morphological measurements of each adult that eclosed by placing live flies under CO_2 anesthesia and viewing a video image digitized into a computer. Using the program NIH Image (ver. 1.59, National Institutes of Health, Bethesda, MD), I measured eye-span between the outer edges of the ommatidia, body length from head to wing tip, and thorax width to the nearest 0.01 mm at a resolution of 50 pixels/mm. Subsequent to the morphological measurements, adult flies were housed in single-sex plastic cages (12 x 16 x 13.5 cm) lined with moist cotton and blotting paper until they were used in the gonad development and sperm transfer experiments.

Gonad development

Starting on the third day after eclosion, I anesthetized male *C. dalmanni* with CO_2 and removed the paired testes onto a slide with several drops of PBS buffer (pH = 7.4). NIH Image was used to measure the area of each testis at 40x magnification. These measurements were then averaged to obtain a single value of testis area for each individual. Dissections were performed for a total of 10 age classes, separated by three-day intervals, up to an age of thirty days. Testis development was recorded for 62 acetone control, 45 medium JH, and 62 high JH males. Since flies treated with acetone did not show any difference in eclosion success or development time (see Results), these flies are assumed to be comparable to untreated individuals, and a separate untreated control was not used.

Female *C. dalmanni* were dissected starting on the ninth day after eclosion. I anesthetized females with CO_2 and removed the female reproductive tract onto a slide with PBS buffer as described above. For each female, I counted the number of fully

elongated eggs present in the ovaries. Dissections were performed for a total of 8 age

classes, separated by three-day intervals, up to an age of thirty days. Mature eggs

were counted for 30 acetone control, 38 medium JH, and 23 high JH females.

Sperm transfer

Sperm transfer by *C. dalmanni* was quantified by counting sperm within

females' spermathecae after copulation. Since age influences sperm production

within testes (Wilkinson and Sanchez 2001) and males reach sexual maturity at 25

days of age (Baker et al. 2003), confounding effects of age were eliminated by using

only 30-day old males. Single virgin males of the acetone control ($n = 23$) or high JH

($n = 20$) treatments were introduced into a small plastic cage (9 x 11 x 9 cm) with

three sexually mature untreated virgin females. Each male was allowed to copulate

once with each of two of these females. Copulations were timed to the nearest second

to ensure that they lasted at least 40 seconds, the minimum duration required for

sperm transfer (Lorch et al. 1993). The mated females were then removed from the

cage and dissected within 1-3 hours of copulation termination. Each female was

dissected under CO_2 anesthesia by placing the tip of the abdomen on a glass side and

removing the reproductive tract into a drop of PBS. After isolating the spermathecae,

I used the Live/Dead Sperm Viability Kit (Molecular Probes L – 7011) to stain stored

sperm. I added 15 µl of dye solution (prepared as described in Fry and Wilkinson

2004) to the spermathecae and gently crushed them under a cover slip to release the

sperm and mix them with the dye solution. I then counted sperm at 200x

magnification under ultraviolet light using a Nikon Eclipse E600 microscope fitted

with two fluorescence filter cubes (B-2E/C and G-2E/C from Nikon). Using these

filter cubes, I counted the total number of red (inviable) and green (viable) sperm

present in each female's spermathecae. Sperm counts for each female were averaged

to obtain a single value for each male. Following the sperm counts, males were

dissected to obtain a measurement of average testis size as described above. These

measurements are included in the gonad development experiment.

Age at sexual maturity

Both male and female *C. dalmanni* exhibit delayed sexual maturity, with

males reaching maturity 25 days after eclosion, and females at 22 days after eclosion

(Baker et al. 2003). To determine if larval JH treatments alter the time required to

reach sexual maturity, I housed immature high JH and acetone control flies with three

sexually mature untreated virgins of the opposite sex. Treated flies for this

experiment did not originate from the morphological measurement study; instead,

additional larvae were treated as previously described.

Female *C. dalmanni* were housed with males starting on day 17, and males

were housed with females starting on day 19. Breeding cups containing

approximately 30 mL of corn medium were changed daily until day 30, or until the

fly died. In every case where an individual died before day 30, sexual maturity was

reached prior to death, so these flies were retained in the data set. Breeding cups

were incubated as described previously, and both pupae and eclosing adults were

counted within each cup. Age at sexual maturity was defined as the number of days

between eclosion and the first day on which fertilized eggs were oviposited. Not all

flies tested were fertile; the following sample sizes indicate the number of fertile flies with the total tested in parentheses: acetone control females, $n = 18$ (23); high JH females, $n = 12$ (15); acetone control males, $n = 18$ (18); high JH males, $n = 18$ (24).

Statistical analyses

All analyses were performed using JMP version 5.0 (SAS Institute, Cary, NC). Transformations were applied as necessary to achieve normality. All pairwise comparisons are post-hoc Fisher's PLSD tests with Bonferroni corrections for multiple tests where needed. Non-significant interactions were removed from the models unless otherwise indicated. Descriptive statistics are expressed as mean ± SE.

RESULTS

Pupal development time and eclosion success

Treatment with the JH agonist methoprene resulted in a dose-dependant effect on eclosion success (Fig. 5a). A contingency test shows that individuals treated with higher concentrations of methoprene were significantly less likely to eclose ($\chi^2 = 13.57$, df = 3, $P = 0.0035$). JH treatment also increased the number of days that elapsed between pupation and eclosion; an ANOVA revealed a significant effect of sex ($F_{1, 304} = 45.16$, $P < 0.0001$) and of hormone treatment ($F_{3, 304} = 19.62$, $P < 0.0001$) but no significant interaction ($F_{3, 304} = 1.12$, $P = 0.34$). In each treatment group, males took longer to develop than females (Fig. 5b). Pairwise comparisons of hormone treatments revealed significant differences in development times. Every comparison was significant at $P < 0.001$ except for acetone vs. untreated controls (P

46

= 0.61), thereby indicating that acetone treatments alone do not appear to affect stalk-eyed fly development. In addition, there was no delay in the onset of pupation for individuals treated with JH, nor were there any obvious malformations of the pupal cuticle.

Adult morphology

In addition to affecting eclosion success and development time, JH treatments altered the mean body size of flies that did eclose. An ANOVA on body length revealed a significant effect of sex ($F_{1, 802} = 9.23$, $P = 0.0025$) and hormone treatment ($F_{2, 802} = 50.39$, $P < 0.0001$), as well as a significant interaction between them ($F_{2, 802} = 5.08$, $P = 0.0064$). Because acetone treatments alone did not have any apparent effect on development, I combined data for untreated and acetone individuals into a single group ("pooled control"). For both sexes, high JH treatments reduced the mean body length of eclosing adults (Table 3). Pairwise comparisons confirmed that body length differed significantly for both pooled control vs. high JH ($P < 0.0001$) and high JH vs. medium JH ($P < 0.0001$) comparisons, but not for pooled control vs. medium JH ($P = 0.31$).

Since hormone treatments created additional variation in body size, I examined JH effects on eye-span using an ANCOVA with body length as a covariate. The full model revealed a significant treatment by sex interaction ($F_{2, 796} = 13.50$, $P < 0.0001$), indicating that males and females responded differently to JH applications. I therefore tested males and females separately. These ANCOVAs show that eye-span allometry, as indicated by significant treatment by body size interactions, changed in

47

males but not females (Table 4). Treatment with high JH increased eye-span in small-bodied males (Fig. 6a), but decreased eye-span across body sizes in females (Fig. 6b).

<p style="text-align:center">*Gonad development*</p>

The mean testis area of males in each treatment group increased throughout the first 30 days of adulthood but showed a tendency to level off over time (Fig. 7a). In order to account for the asymptotic relationship between testis size and age, I transformed the measurements using ln (testis +1). An ANCOVA on transformed testis size revealed significant effects of age, body length, and hormone treatment, as well as a significant interaction between treatment and body length (Table 5). Absolute mean testis area at 30 days shows that high JH males have testes approximately 2/3 the size of medium JH and control males at sexual maturity (Fig. 7a).

Counts of mature eggs within female reproductive tracts were not distributed normally due to zeros in the data set for younger ages. Therefore, all numbers of mature eggs were transformed using ln (eggs +1). An ANCOVA demonstrates that transformed egg counts were significantly affected by age, but not by hormone treatments or body length (Table 5). The number of mature eggs in female ovaries increased throughout the first 30 days of adulthood, but, like testis size, showed a tendency to level off near sexual maturity (Fig. 7b).

<p style="text-align:center">*Sperm transfer*</p>

Although I observed two full-length copulations for each male, not all copulations resulted in sperm transfer. Ten control and 5 high JH males achieved only one successful sperm transfer. An ANOVA on the number of sperm in females' spermathecae revealed a significant effect of hormone treatment ($F_{1, 40} = 13.47$, $P = 0.0007$) and transformed testis size ($F_{1, 40} = 9.99$, $P = 0.003$). High JH males passed approximately 2/3 as many sperm to females as control males (Fig. 8a). Viability staining revealed that all sperm examined within the females' spermathecae were viable. In addition, copulation duration did not differ between high JH and control males ($F_{1, 41} = 2.60$, $P = 0.12$).

Age at sexual maturity

An ANOVA on the number of days required to reach sexual maturity demonstrated a significant effect of sex ($F_{1, 62} = 185.9$, $P < 0.0001$) but not hormone treatments ($F_{1, 62} = 0.82$, $P = 0.37$). Males of both the high JH and control groups required more days to reach maturity than females (Fig. 8b). When the treatments are combined, males require an average of 24.0 ± 0.29 days and females require 18.8 ± 0.23 days to become sexually mature.

DISCUSSION

The results presented here indicate that exogenous JH applied during development alters the size of a secondary sexual trait in stalk-eyed flies. Males responded to treatment with an increase in relative eye-span which was most pronounced in smaller-bodied individuals. Conversely, females demonstrated a

reduction in eye-span across all body sizes. In addition, JH treatment prolonged
pupal development and lowered eclosion success. Testis growth and the number of
sperm transferred to females were reduced in adult males, but egg maturation was
unaffected in adult females. Adults of both sexes showed no difference in the time
required to reach sexual maturity after larval JH treatments. These results are
discussed in the context of hormone-mediated trade-offs between developing body
parts.

JH alters the growth of eye-stalks

Applications of high JH to final instar *C. dalmanni* larvae altered the eye-span
of eclosing adults, while the medium JH and control treatments had no effect on eye-
span. Stalk-eyed flies may therefore have a threshold for sensitivity to JH, as
documented in studies of polyphenic insects (Dingle and Winchell 1997; Moczek and
Nijhout 2002). The lack of dose-dependence may also be explained by the 10-fold
difference in the two concentrations of methoprene; it is possible that intermediate
dosages may be able to elicit a graded response. Males responded to treatment with
an increase in eye-span relative to body size while females demonstrated a reduction
(Fig. 6). In addition, the effect on male eye-span altered the slope of the allometry
such that males of smaller body sizes had comparatively greater increases in eye-
span. The shift in eye-span allometry may be partially explained by the mortality of
larger males, an effect that truncated the range of body sizes for the high JH treatment
(Fig. 6a). Alternatively, the increase in developmental time may lead to a reduction
in body size while eye-span remains largely unaffected, resulting in an apparent
increase in relative eye-span. However, this seems less likely given that eye-stalk

development continues throughout the pupal phase (Buschbeck et al. 2001). A third possibility is that larger males may already be at the limit of trait expression due to some developmental constraint (e.g. Knell et al. 2004) such that additional hormone had no effect.

Sex-specific responses to JH treatments may reflect the ability of imaginal discs to be regulated relatively autonomously within developing larvae (Nijhout 1994; Emlen and Allen 2004). For example, the growth of discs in *Drosophila* occurs primarily by cell proliferation (Johnston and Gallant 2002), and changes in rates or amounts of proliferation (and therefore in final trait sizes) probably evolved via shifts in the relative sensitivities of individual discs to circulating hormone levels (Truman and Riddiford 1999, 2002). Recent reviews have also stressed that variation in disc proliferation and differentiation due to changes in thresholds to both JH and ecdysone may act as a general mechanism underlying allometric relationships (Stern and Emlen 1999; Emlen and Allen 2004). In stalk-eyed flies, the eye antennal imaginal discs from which eye-stalks originate likely differ between the sexes in either the density of JH receptors, the sensitivity of individual receptors to JH, or both. The apparent difference in JH sensitivity between the sexes may reflect the fact that male eye-span has been a frequent target of selection within the Diopsidae (Wilkinson and Dodson 1997; Wilkinson and Taper 1999; Baker and Wilkinson 2001). Genetic changes that alter the threshold sensitivity of imaginal discs to JH may therefore contribute to the evolution of exaggerated eye-span. While the reduction of eye-span in females was unexpected, sex-specific differences in sensitivity to JH are consistent with models of

hormone-mediated changes in trait scaling (Stern and Emlen 1999; Emlen and Nijhout 2000; Emlen and Allen 2004).

JH prolongs pupal development and reduces eclosion success

Prior to pupal commitment, applications of JH can prolong the larval stage and delay the onset of pupation (Nijhout 1994; Emlen and Nijhout 1999). At the conclusion of the larval phase, JH levels must be either absent or below a threshold to achieve a normal molt (Rountree and Bollenbacher 1986; Bollenbacher 1988; Nijhout 1994). Exogenous JH applied to stalk-eyed fly larvae after the gut purge results in a normal molt to the pupal stage with no delay in the onset of pupation. This result is consistent with the endocrine control of metamorphosis and with experimental evidence from *O. taurus* beetles (Emlen and Nijhout 1999). While exogenous applications of JH did not delay the onset of pupation, the length of the pupal phase was extended by about one full day in high JH flies. In all treatment groups, males took longer to complete development than females (Fig. 5b). Sex-specific patterns of development time are also present in many *Drosophila* species, and artificial selection experiments in *D. hydei* suggest that the increased duration of developmentin males is due to additional investment of larval resources in testes (Pitnick and Miller 2000).

Given that the testes of *C. dalmanni* are small at eclosion compared to *Drosophila*, an *a priori* prediction might ascribe the increased male development time to the exaggerated eye-stalks. The formation of eye-stalks in the morphologically similar congener *C. whitei* occurs during the first third of the pupal phase and continues until eclosion, when the expansion of the head capsule inflates the stalks

and displaces the eyes laterally (Buschbeck et al. 2001). In addition, *C. dalmanni* artificially selected for large eye-span take about a day longer to develop than unselected flies (Wilkinson 1993). The finding that JH increased both development time and eye-span in males is consistent with this prediction. However, development time was also increased in females, which demonstrated a reduction in eye-span. Interestingly, selection on testis length in male *D. hydei* increased development time in females as well (Pitnick and Miller 2000), suggesting a correlation between male and female developmental programs irrespective of morphology. Evidence from populations of *O. taurus* that differ in thresholds for horn production also indicates that the time required to complete development may not be directly coupled to the production of physical traits (Moczek and Nijhout 2002). The effect of hormone treatment on development time in stalk-eyed flies may therefore be a reflection of the pleiotropic nature of JH action rather than an indication of increased investment in eye-span.

In addition to affecting eye-span and development time, applications of high JH caused the greatest pupal mortality. The reduction in body length caused by this treatment suggests that the larger individuals failed to eclose. Size-dependent mortality is especially evident in males, where no adults over 7.00 mm in body length eclosed (Fig. 6a). In *Drosophila*, JH agonists are lethal when applied in high concentrations near the onset of metamorphosis (Postlethwait 1974; Bouchard and Wilson 1987; Restifo and Wilson 1998; Wilson 2004). Lethality occurs relatively late during metamorphosis, often just prior to emergence, and does not typically prevent normal pupation (Postlethwait 1974; Riddiford and Ashburner 1991). Death

appears to result from a variety of relatively subtle internal defects in the nervous system and musculature which are mediated by JH-induced disruptions in ecdysone-regulated transcription factors (Restifo and Wilson 1998). The exact cause of death in stalk-eyed fly pupae previously treated with methoprene is unknown; however, dissections of pupae that never eclosed revealed pharate adults that appeared to be well developed with no obvious external deformities (unpublished data).

JH reduces testis growth and the number of sperm transferred

The testes of stalk-eyed flies in all treatment groups were small shortly after eclosion and grew rapidly over the first 30 days of adulthood (Fig. 7a). Medium JH and control males had similar testis sizes at each age class, while the high JH flies had consistently smaller testes. Since male *C. dalmanni* reach sexual maturity prior to day 30 (Baker et al. 2003), the smaller testes of high JH flies may be detrimental to reproductive fitness, especially given the high frequency of mating in this species (Wilkinson et al. 2003). It is unknown to what extent testis growth in treated individuals continues after day 30, but other studies of *C. dalmanni* have shown that testis growth tends to level off with age (Baker et al. 2003) and may demonstrate some change up to 80 days (Wilkinson et al. 2005). An examination of Fig. 7a suggests that testis growth is approaching an upper maximum in each treatment group, a pattern which is consistent with testis growth in *Drosophila* (Pitnick and Miller 2000). In contrast to gonad development in males, the production of mature eggs by females did not demonstrate any relationship with hormone treatments (Fig. 7b).

Across *Drosophila* species, testis length is positively correlated with both

sperm production and the number of sperm transferred to females during copulation

(Pitnick 1996). Such a relationship also appears to exist in *C. dalmanni*, where high

JH males had both significantly smaller testes and reduced sperm counts. Treated

males passed less than half as many sperm to females, on average, as control males.

Although females may eject spermatophores before they are fully emptied (Kotrba

1991), this is unlikely to account for the magnitude of the difference between treated

and control males. Control males transferred approximately 66 sperm in an ejaculate,

which is nearly twice the number of sperm transferred by its congener *C. whitei* (~

34) (Fry and Wilkinson 2004). These two species also vary in the degree of multiple

mating by males (Wilkinson et al. 2003), and comparisons across stalk-eyed fly

species may reveal to what extent sperm transfer is influenced by testis size.

JH does not affect age at sexual maturity

Age at sexual maturity is a key life history trait that varies greatly across taxa

(Stearns 1992) and, in *Drosophila*, is positively correlated with testis size (Pitnick et

al. 1995). Given the relationship between maturity and testis size in other Diptera, it

is perhaps surprising that the substantial reduction in testis size in high JH males did

not result in any significant delay in sexual maturity. Males of both treated and

control groups took approximately 24 days to reach maturity, while females required

approximately 19 days. Delayed male maturity was also observed in another study of

C. dalmanni (Baker et al. 2003) and is evident in 24 out of 42 species of *Drosophila*

(Pitnick et al. 1995). Unlike *Drosophila*, delayed maturity in *C. dalmanni* was better

explained by the size of male accessory glands than by testis size (Baker et al. 2003).

The results presented here similarly indicate a lack of a relationship between testis

size and sexual maturity. Accessory glands were not measured in this study;

however, given the importance of accessory gland proteins in determining

reproductive success in these and other flies (Chapman 2001; Wolfner 2002; Fry and

Wilkinson 2004), it would be interesting to determine what relationship, if any, exists

between eye-span and these glands. The relative sizes of primary and secondary

sexual traits may prove to be highly variable: data from field-caught rhinoceros

beetles indicate that population-level variation between an exaggerated trait and

genitalia can evolve rapidly (Kawano 2004). Interestingly, experiments with

Drosophila also suggest that *Methoprene-tolerant* (*Met*), a gene which codes for a

putative JH receptor, regulates the production of protein in male accessory glands

(Wilson et al. 2003).

Implications for hormone-mediated trade-offs

In addition to coordinating development and metamorphosis during the larval

and pupal phases, JH also regulates reproduction in the adult phase. For example, JH

has been shown to initiate ovarian maturation and the onset of sexual behavior in

females (Manning 1966; Saunders et al. 1990; Zhou et al. 2000). In males,

exogenous JH can induce pheromone release and early sexual maturity (Yin et al.

1999; Teal et al. 2000). Hormones in general and JH in particular have increasingly

been implicated in suites of life-history trade-offs involving reproduction (see Finch

and Rose 1995 and references therein). For example, JH mediates the morph-specific

56

reproduction of wing-polyphenic crickets, where there is a trade-off between wing length and ovary size (Zera et al. 1998a; Cisper et al. 2000). In *Drosophila*, JH enhances reproduction but increases susceptibility to stress (Salmon et al. 2001) and decreases lifespan (Tatar et al. 2001). Experiments with *Drosophila Met* mutants also suggest that the pleiotropic effects of this gene on life history traits may provide a causal link between JH and trade-offs (Flatt and Kawecki 2004). Since JH typically enhances adult reproduction, the negative effect of JH applications on testis size and the lack of an effect on sexual maturity in stalk-eyed flies may at first seem counterintuitive. However, the preceding examples all measured or altered JH titers in adults. The effect of JH on reproduction is likely dependent on the developmental phase during which it is administered. Here, applications of high JH to larval stalk-eyed fly males increased eye-span but reduced the testis size of adults and lowered the number of sperm transferred to females. None of the hormone treatments applied to females caused a change in egg maturation. Taken together, these results suggest that JH mediates a trade-off between a primary sexual trait and a secondary sexual trait, and support the emerging role of JH as a key mediator of trade-offs.

Given that JH levels have to drop below a threshold to achieve normal pupation (Nijhout 1994), it appears unlikely that increased titers of JH from larval applications persisted throughout adulthood. Furthermore, enhanced titers in adults would be expected to have the opposite effect on testes, either causing early maturation or an enhanced rate of growth. However, JH may indirectly mediate the allocation of resources to eye-span at the expense of gonads. Several lines of evidence support a resource allocation hypothesis for the dual effect of JH on eye-

span and gonads. Eye-span is condition-dependent, and adult eye-span allometry depends upon the amount of resources acquired during the larval phase (David et al. 1998, 2000; Cotton et al. 2004a). The majority of imaginal disc growth in holometabolous insects occurs after the acquisition of resources (Nijhout 1994; Emlen and Nijhout 2000; Emlen and Allen 2004). Hence, resource allocation decisions must be made in a closed system, yielding an environment more favorable for trade-offs than that of hemimetabolous insects (Tomkins et al. 2005). In addition, the development of exaggerated traits such as eye-stalks should make trade-offs more likely, as the production of these morphologies presents a potentially significant drain on resources (Emlen and Nijhout 2000). A link between JH and resource allocation is also supported by evidence that JH titers as well as the size of the corpora allata, the organ that produces and secretes JH, are affected by feeding rate and diet quality (Osorio et al. 1998; Trumbo and Robinson 2004).

Since JH applications induced males to produce larger eye-stalks relative to body size, the allocation of resources to eye-span may reduce the amount of resources available for gonad development. When adult *C. dalmanni* are reared on nutritionally restricted diets, testis size is significantly reduced (Baker et al. 2003). Given that adult *C. dalmanni* in this study were reared on identical, high-quality diets, it not immediately evident why testis sizes were consistently smaller over 30 days for high JH males. The trajectory of testis growth may be predicted by initial size at eclosion, as high JH males had comparatively smaller testes at younger age classes. Alternatively, the increase in relative eye-span may induce energetic costs that continue into adulthood, thereby limiting additional expenditure on reproduction. For

58

example, *C. whitei* males show a reduction in aerial performance consistent with costs of eye-stalks on flight (Swallow et al. 2000). Trade-offs between an exaggerated trait and male genitalia have also been demonstrated in *O. taurus*, although the extent to which these are regulated by JH remains unknown (Moczek and Nijhout 2004).

The apparent trade-off between eye-span and testes suggests that competition between body parts during stalk-eyed fly development may have long-term consequences for adult reproduction. However, it should be stressed that negative associations are not necessarily indicative of resource allocation trade-offs. For example, negative relationships between life-history traits may arise due to molecular signaling pathways, independent of any evidence supporting the involvement of resources (Leroi 2001, but see Barnes and Partridge 2003). While the results presented here are consistent with resource allocation trade-offs, they do not conclusively demonstrate a causal relationship. Additional experiments focus on manipulating diet quality and will help evaluate the impact of resources on morphological trade-offs in these flies. JH applications reveal that eye-span is sensitive to this hormone, indicating that traits with continuous scaling relationships may be hormonally regulated in a fashion similar to that proposed for discontinuously scaling traits (Nijhout 2003). The molecular mechanism underlying the effects of JH on morphology remains largely undetermined, but recent studies have suggested that JH works via a lipid signaling system that can act in both transcriptional regulation and signal transduction in imaginal discs (Wheeler and Nijhout 2003; Emlen and Allen 2004). Such a global mechanism may explain why JH is capable of mediating

the development of a wide diversity of insect forms, although the extent to which this

regulation governs trade-offs remains an open question.

TABLE 3. Absolute (mm) body lengths (mean ± SE) of adult stalk-eyed flies after larval methoprene treatments. JH = Juvenile hormone.

Hormone Treatment	males	females
Pooled Control	6.75 ± 0.032	6.56 ± 0.032
Medium JH	6.76 ± 0.039	6.64 ± 0.035
High JH	6.32 ± 0.031	6.36 ± 0.046

TABLE 4. The results of an ANCOVA on eye-span with body length as a covariate.

Source of variation	df	F	P
Females			
Hormone treatment	2	18.42	<0.0001
Body length	1	266.47	<0.0001
Treatment x body length	2	0.63	0.53
Error	350		
Males			
Hormone treatment	2	1.70	0.18
Body length	1	770.82	< 0.0001
Treatment x body length	2	11.66	< 0.0001
Error	446		

TABLE 5. The results of an ANCOVA on transformed testis size and egg counts with body length as a covariate.

Source of variation	df	*F*	*P*
Ln (testis +1)			
Hormone treatment	2	48.76	<0.0001
Body length	1	7.87	0.0056
Age	1	438.41	<0.0001
Treatment x body length	2	4.51	0.012
Error	162		
Ln (eggs +1)			
Hormone treatment	2	0.123	0.88
Body length	1	0.011	0.92
Age	1	126.93	<0.0001
Error	86		

FIG. 5. (a) Percentage of adults successfully eclosing following larval treatment with methoprene. Numbers on each bar indicate the total number of treated individuals. (b) Average duration of the pupal phase following larval treatments with methoprene. Error bars indicate ± SE and bar colors indicate males (gray) and females (white).

FIG. 6. Eye-span of adult males (a) and females (b) following larval methoprene treatments. Symbols and regression line patterns represent treatment types as indicated on the figure.

FIG. 7. Mean testis area of adult males (a) and counts of mature eggs in female ovaries (b) following larval methoprene treatments. Symbols and line patterns represent treatment types as indicated on the figure. Error bars indicate ± SE.

FIG. 8. (a) Least squares mean (± SE) numbers of sperm transferred to females by males treated with methoprene as larvae. (b) Least squares mean (± SE) age at sexual maturity for males (gray) and females (white).

Body length (mm)

Eye-span (mm)

(a) Males

(b) Females

Pooled Control
Medium JH
High JH
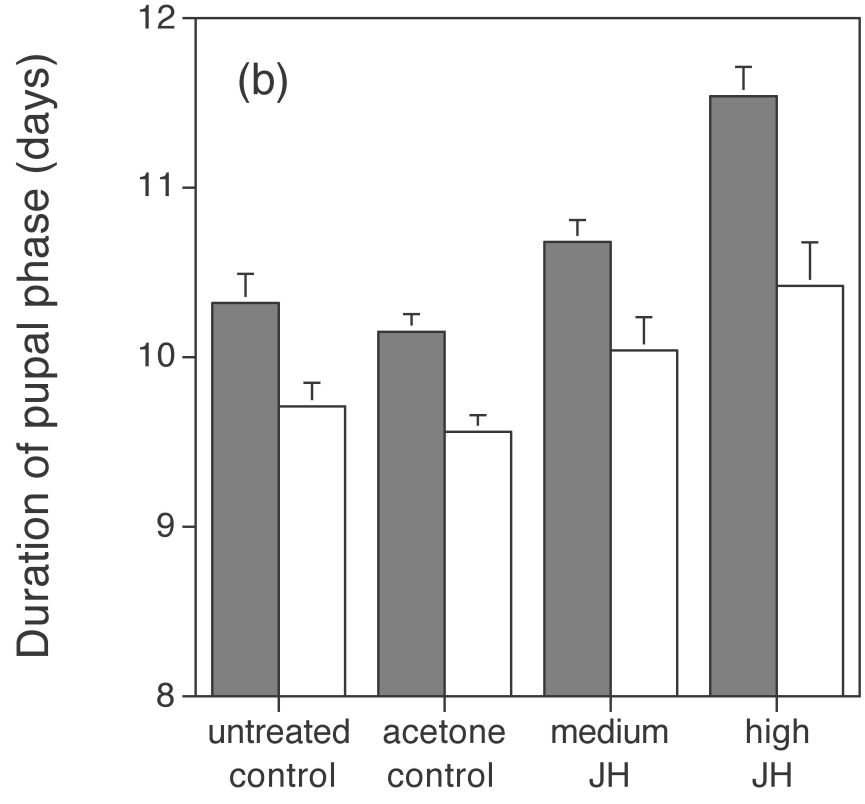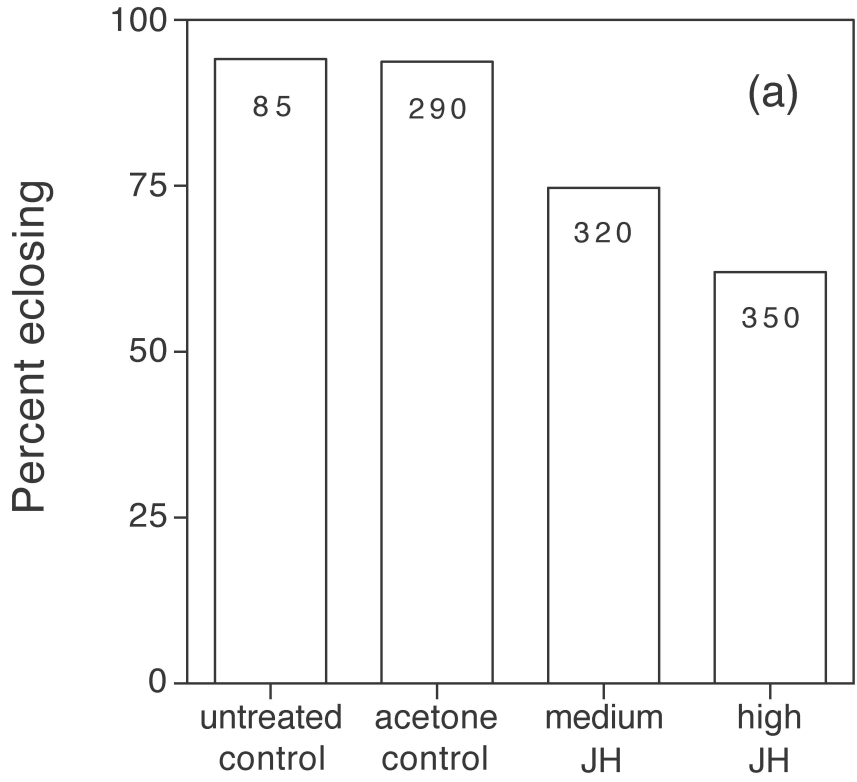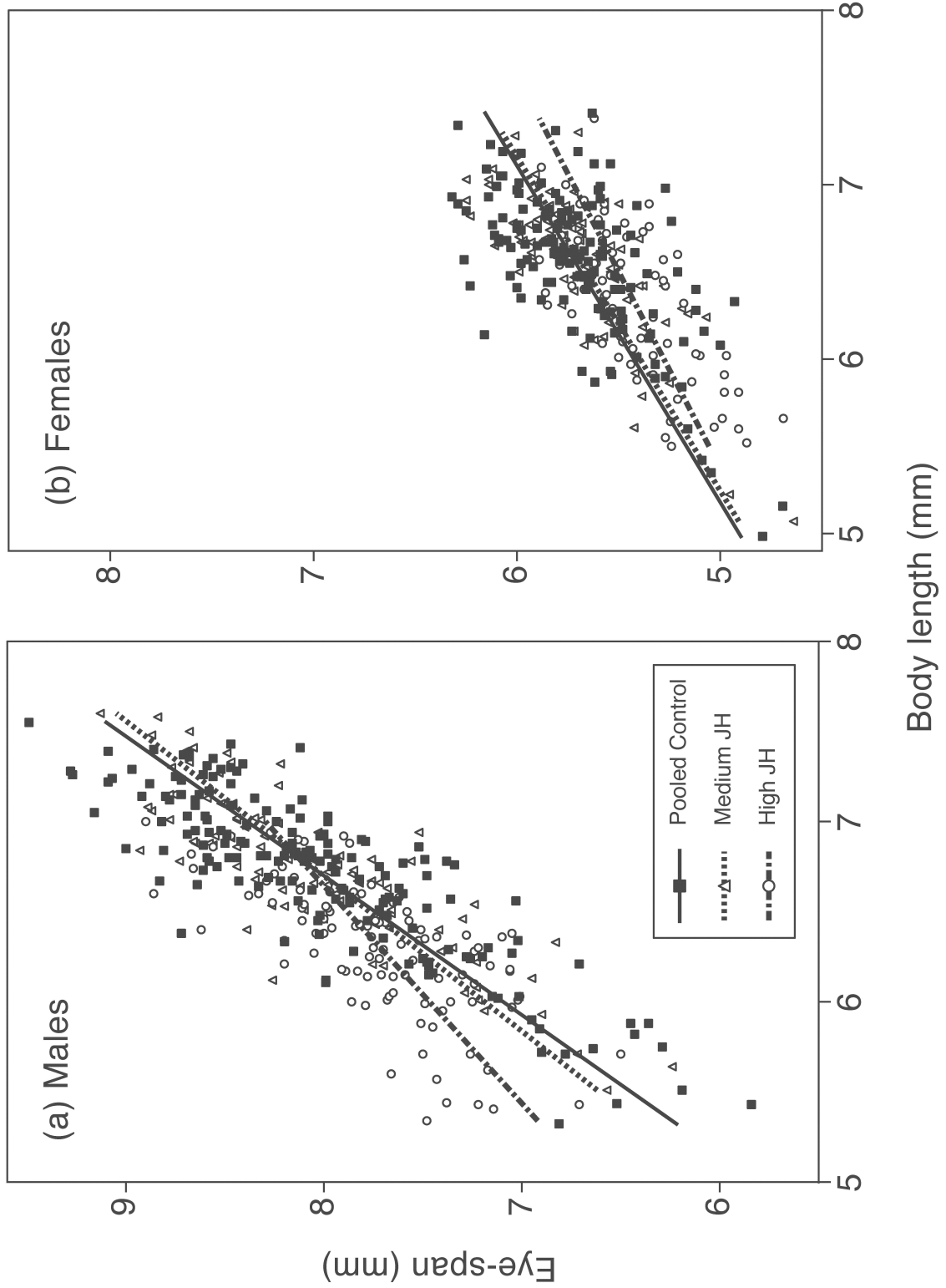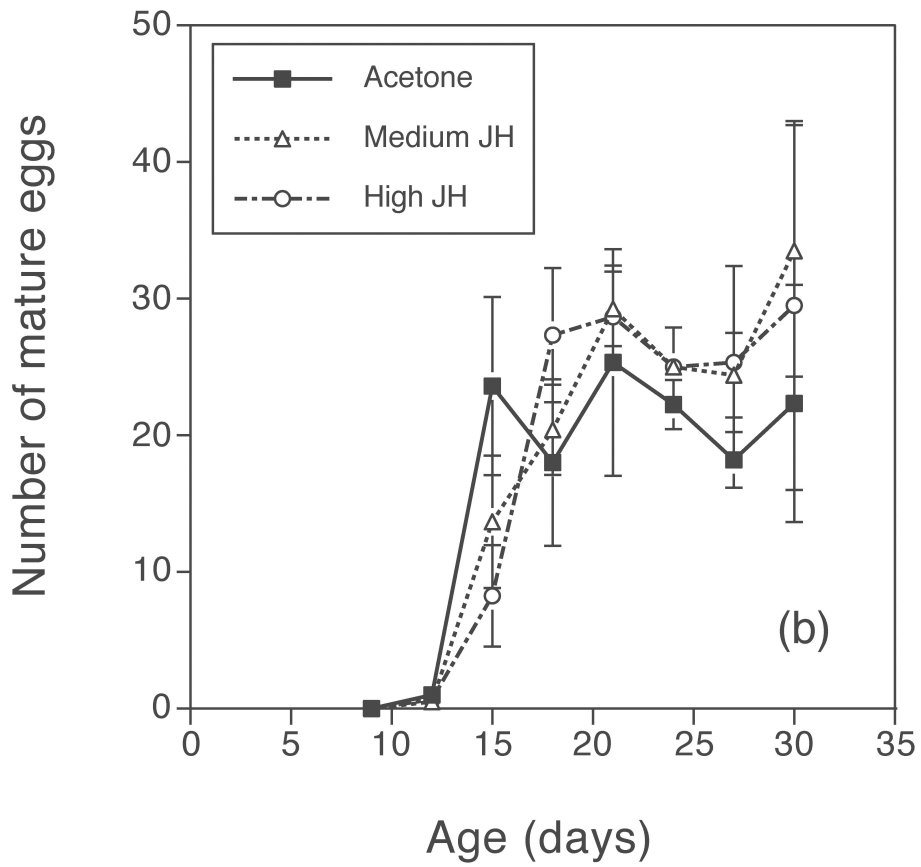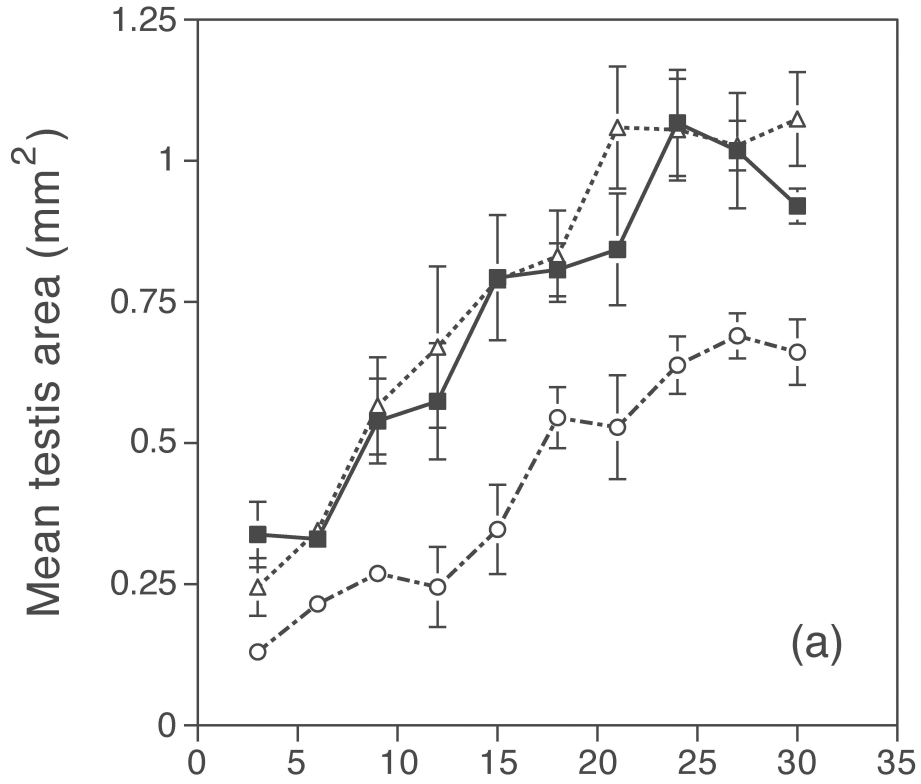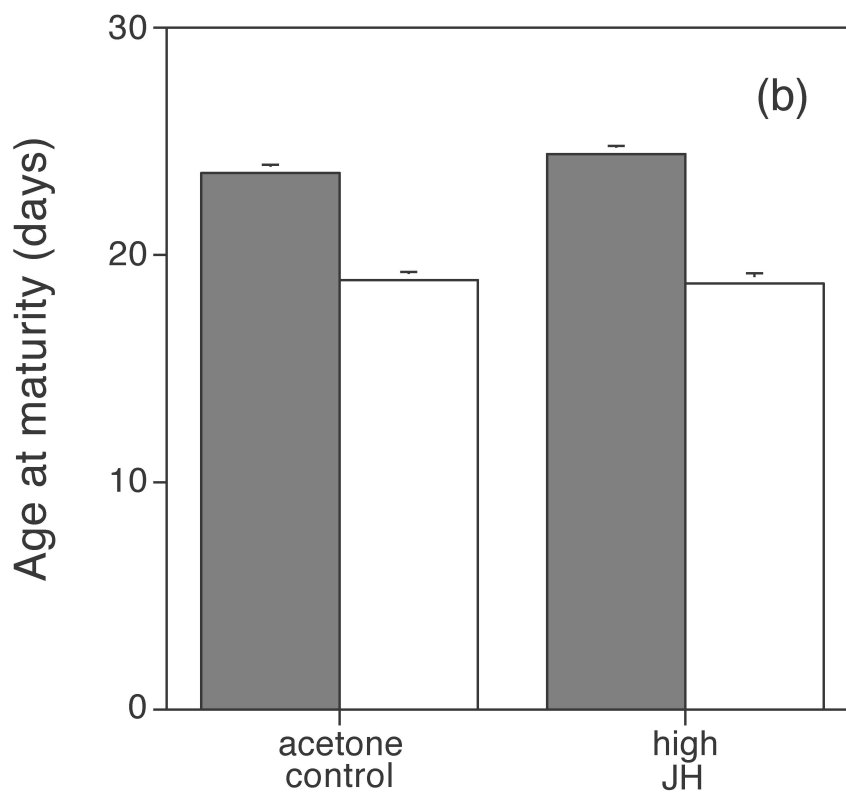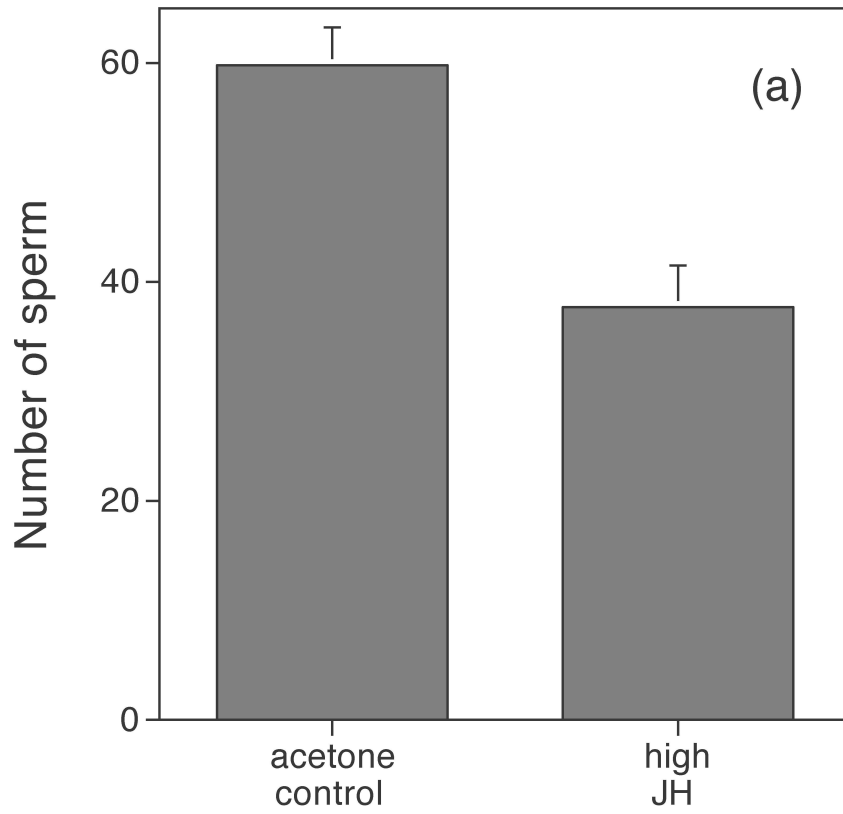
CHAPTER 3: EFFECTS OF LARVAL NUTRITION ON ADULT MORPHOLOGY AND GONAD DEVELOPMENT IN STALK-EYED FLIES THAT DIFFER IN EYE-SPAN

ABSTRACT

Resource allocation trade-offs during development have the potential to dramatically affect insect morphology. Furthermore, insects with condition-dependent ornaments are hypothesized to experience trade-offs resulting from the allocation of resources to an exaggerated secondary sexual trait. Here, I test this prediction using two related species of stalk-eyed flies that differ in the degree of eye-span exaggeration. Resources were manipulated by rearing *Cyrtodiopsis dalmanni* (sexually dimorphic) and *Cyrtodiopsis quinqueguttata* (sexually monomorphic) in food of varying nutritional quality. Measurements of emerging adults indicate that diet quality affected eye-span in male *C. dalmanni* but did not induce any trade-offs with other external morphological traits, including eye-bulb size, eye-stalk width, and wing length. Adult gonad size, however, was significantly reduced in poor diets in male *C. dalmanni*, suggesting that environmental stress caused males to allocate resources to eye-span at the expense of testes. Furthermore, this effect persisted at least 30 days into adulthood, revealing a trade-off across a developmental boundary. Neither female *C. dalmanni* nor either sex of *C. quinqueguttata* showed evidence of trade-offs between any traits. These results are consistent with predictions made by sexual selection theory and suggest a potential cost of developing exaggerated eye-span.

The allocation of resources within an organism is a nearly universal process that affects numerous taxa. In any environment where resources are limited, or the time in which to acquire them is limited, an organism must allocate resources to one trait at the expense of one or more competing traits. The resulting trade-offs are a central prediction of life history theory (Roff 1992; Stearns 1992). Physiological trade-offs between adult traits such as current and future reproduction have been studied extensively in model systems including mammals, birds, lizards, insects, and flowering plants (Clutton-Brock et al. 1982; Roff 1992; Stearns 1992; Sinervo and Svensson 1998; Zera and Harshman 2001). However, in addition to physiological trade-offs during adulthood, morphological trade-offs may arise during development. In organisms where adult body size is fixed, resource allocation during development may have profound consequences for adult morphology. Holometabolous insects provide a system where resources are acquired during the larval phase, but the bulk of phenotypic trait development occurs when imaginal discs undergo exponential growth after the cessation of feeding (Nijhout 1994; Truman and Riddiford 1999, 2002). Resource allocation decisions must therefore be made in a closed system with finite materials, creating an environment favorable for trade-offs.

Given that holometabolous insects go through multiple life-history stages, the allocation of a finite pool of resources during one stage is likely to affect subsequent stages. For example, selection for faster larval development in *Drosophila* results in decreased adult body size at eclosion and a reduction in fecundity (Nunney 1996; Prasad et al. 2001). In caddis flies, increased energy expenditure in larval defense

results in reduced relative wing size which may compromise flight efficiency in adults (Stevens et al. 1999). Increased investment in mandibles in stag beetles also induces a trade-off with wings (Kawano 1997). Larval food limitation causes a reduction in body size and reduced fecundity in brine flies (Collins 1980) and a trade-off between body size and wing length in *Speyeria* butterflies (Boggs and Freeman 2005). Conversely, the relaxation of nutritive demands during development can preferentially increase allocation to morphological traits. Experimental removal of butterfly hindwing imaginal discs causes compensatory growth of legs and forewings (Klingenberg and Nijhout 1998; Nijhout and Emlen 1998). In horned beetles, surgical ablation of the genital discs from which male genitalia arise results in disproportionately large horns (Moczek and Nijhout 2004). The absolute and relative sizes of morphological traits therefore appear to be regulated in part by interactions with other competing structures (Nijhout and Emlen 1998).

In addition to empirical studies that measure physical trade-offs, competition between developing body parts is predicted by theoretical models of imaginal disc growth (Nijhout and Wheeler 1996). Several studies have suggested that trade-offs result from inter- or intra-disc competition for a hemolymph-borne growth factor or hormone (Klingenberg and Nijhout 1998; Moczek and Nijhout 2004; Tomkins et al. 2005). Juvenile hormone (JH), a regulator of metamorphosis, has recently been utilized to alter growth trajectories of specific body parts in order to change patterns of resource allocation and induce developmental trade-offs (Nijhout and Emlen 1998; Zera et al. 1998a). A link between JH and resource allocation is supported by evidence that JH titers as well as the size of the corpora allata, the organs that produce

71

and secrete JH, are affected by feeding rate and diet quality (deWilde and Beetsma 1982; Ono 1982; Osorio et al. 1998; Trumbo and Robinson 2004). Recent evidence also suggests that interactions between JH and nutrition influence the proliferation and differentiation of imaginal discs, and thus the final size of adult traits (Emlen and Allen 2004).

If JH directly or indirectly influences resource allocation, a complementary approach to hormone applications is the manipulation of the resources themselves. The storage and expenditure of resources influences condition-dependant signaling, and therefore plays a role in sexual selection (Tomkins et al. 2004). Exaggerated secondary sexual traits are predicted to be sensitive to condition, thereby serving as reliable signals of quality (Zahavi 1975; Iwasa et al. 1991; Andersson 1994). Empirical evidence from *Onthophagus* beetles supports a role for condition in shaping the morphology of a sexually selected trait (elongated horns), where variations in food availability and quality affect both the absolute size of horns and horn allometry (Emlen 1994, 1997). If the development of exaggerated traits requires a significant portion of available resources, holometabolous insects that bear such traits may be especially likely to experience trade-offs with other competing structures. This appears to be true for *Onthophagus* beetles, which demonstrate trade-offs between horns and eyes (Nijhout and Emlen 1998; Emlen 2001), antennae (Emlen 2001), wings (Emlen 2001; Tomkins et al. 2005), and genitals (Moczek and Nijhout 2004). Furthermore, the 'genic capture' (Rowe and Houle 1996) hypothesis predicts that condition-dependent traits should be more likely to experience trade-offs than traits which are insensitive to condition (Tomkins et al. 2004).

Here, I test this prediction using two species of a holometabolous insect, the stalk-eyed fly (Diptera, Diopsidae), that differ in morphology. *Cyrtodiopsis dalmanni* and *C. quinqueguttata* both have eyes displaced laterally away from the head on elongated "stalks." However, *C. dalmanni* exhibits sexual dimorphism such that male eye-span is greater than their body length and female eye-span is less than their body length (Wilkinson 1993). *C. quinqueguttata* is sexually monomorphic, with males and females having approximately equivalent relative eye-span (Baker and Wilkinson 2001). Male eye-span in *C. dalmanni* is sexually selected via female choice (Wilkinson and Reillo 1994) and male-male competition (Panhuis and Wilkinson 1999). Male eye-span in *C. dalmanni* also displays condition dependence (David et al. 1998, 2000; Cotton et al. 2004a), whereas eye-span in *C. quinqueguttata* appears to be unaffected by condition (Wilkinson and Taper 1999). The elongated eye-stalks of *C. dalmanni* are therefore expected to be more likely to experience trade-offs than the comparatively unexaggerated eye-stalks of *C. quinqueguttata*. To test this prediction, I manipulate larval diet quality and measure the morphology of emerging adults. Differences in resource allocation can be evaluated by examining trade-offs between the physical sizes of different body parts. Since both the eyes and eye-stalks originate from the eye-antennal imaginal disc (Buschbeck et al. 2001; Hurley et al. 2002), I first assess the possibility of within-disc trade-offs between eye-span, eye-stalk width, and eye-bulb area. I then examine potential trade-offs between eye-span and more distant structures: wings and gonads. These results are discussed in the context of differential resource allocation both within and between species.

Fly stocks

C. dalmanni flies used in this study were descended from individuals captured near Ulu Gombak, peninsular Malaysia (3º 54´ N, 101º 26´ E) in August 1999. *C. quinqueguttata* were captured near Bukit Ringit, Malaysia (3º 42´ N, 102º 8´ E) in January 1996. For each species, 10 males and 10 females were housed in a Nalgene cage (45 x 24 x 20 cm) lined with moist cotton and blotting paper to keep humidity elevated. All flies were maintained on a 12 L:12 D cycle at 25º C with at least 75% relative humidity. Adults feed and oviposit on a mixture of pureed corn containing 1% methylparaben as a mold inhibitor (Wilkinson 1993). Plastic cups containing approximately 30 mL of corn puree were changed twice weekly and incubated at the conditions described above. Adults emerging from these cups were returned to cages as necessary to maintain 10 individuals of each sex.

Diet manipulations

Diet quality was varied by replacing a portion of the pureed corn with methyl cellulose (Sigma M-0512), an indigestible food additive (Boots and Begon 1994). Solid methyl cellulose was dissolved in a 1% solution of methylparaben to create a solution of 1% methyl cellulose. The methyl cellulose solution was then mixed with pureed corn to achieve a 2:1 ("medium" quality) and 1:1 ("low" quality) solution of corn: methyl cellulose at a final volume of 30 mL. The control diet ("high" quality) consisted of 30 mL of pureed corn as described above.

Two plastic cups containing identical diet treatments were placed in each cage

at the beginning of the 12 L: 12 D cycle, and removed after 3-6 hours. This ensured

that all eggs oviposited were of comparable developmental stages. Eggs in excess of

30 were removed from each cup prior to incubation to minimize effects of larval

competition for food. Diet treatments were supplied in random order and added to

cages every third day. Adults were additionally supplied with plastic trays of control

corn diet that were changed twice weekly. While females oviposited in all diet

treatments, adults of both sexes preferentially fed on the separately provided control

diet (unpublished data). Diet treatments were incubated as described above and

monitored daily to record time to pupation and egg-to-adult development time. Not

all pupae eclosed; eclosing adults included 127 high, 66 medium, and 94 low diet

quality *C. dalmanni*, and 84 high, 44 medium, and 37 low diet quality *C.

quinqueguttata*. Development times were monitored for a subset of the high diet

quality flies (*C. dalmanni*, $n = 91$; *C. quinqueguttata* , $n = 73$) and all of the medium

and low diet quality flies.

Morphological measurements

After eclosion, I obtained morphological measurements of each adult by

placing live flies under CO_2 anesthesia and viewing a video image digitized into a

computer. Each fly was placed in an identical position, resting on the thoracic and

interorbital spines. Using the program NIH Image (ver. 1.59, National Institutes of

Health, Bethesda, MD), I measured eye-span between the outer edges of the

ommatidia, body length from head to wing tip, and wing length from the point of

insertion to the wing tip. Only one wing was measured on each individual as previous studies have demonstrated that wing length asymmetry is unaffected by larval condition (David et al. 1998; Cotton et al. 2004a). I also measured the width of the eye-stalk immediately adjacent to the interorbital spine and the area of the pigmented optical surface of the eye bulb. All measurements were taken to the nearest 0.01 mm at a resolution of 50 pixels/mm. Following the morphological measurements, a subset of the adult flies were housed in groups of 10 in single-sex plastic cages (12 x 16 x 13.5 cm) lined with moist cotton and blotting paper until they were 30 days old. These flies were supplied solely with the control corn diet.

Gonad measurements

I anesthetized male *C. dalmanni* and *C. quinqueguttata* with CO_2 and removed the paired testes onto a slide with several drops of PBS buffer (pH = 7.4). NIH Image was used to measure the area of each testis at 40x magnification. These measurements were then averaged to obtain a single value of testis area for each individual (*C. dalmanni*: high quality, $n = 17$; medium quality, $n = 16$; low quality, $n = 17$; *C. quinqueguttata*: high quality, $n = 10$; medium quality, $n = 10$; low quality, $n = 10$). I anesthetized females with CO_2 and removed the female reproductive tract onto a slide with PBS buffer as described above. For each female, I counted the number of fully elongated eggs present in the ovaries. I also measured the length of three randomly chosen mature eggs from each individual to the nearest 0.01 mm at 40x magnification (*C. dalmanni*: high quality, $n = 15$; medium quality, $n = 10$; low quality, $n = 16$; *C. quinqueguttata*: high quality, $n = 18$; medium quality, $n = 12$; low

quality, $n = 10$). One *C. dalmanni* female of the low quality diet treatment did not

produce any mature eggs, so this individual was not included in the egg length data

set. All individuals of both sexes were 30 days old to eliminate the variable of age on

gonad development and to ensure that all flies were sexually mature (Baker et al.

2003).

Statistical analyses

All analyses were performed using JMP version 5.0 (SAS Institute, Cary, NC,

USA). Transformations were applied as necessary to achieve normality. All pairwise

comparisons are post-hoc Fisher's PLSD tests and errors are indicated by $\pm$ SE.

Sequential Bonferroni corrections for multiple tests were used where needed. Non-

significant interactions were sequentially removed from the models unless otherwise

indicated.

RESULTS

Development time

Variation in diet quality had no effect on successful eclosion. An ANOVA of

the arcsin squareroot transform of the percent of pupae eclosing in each cup revealed

no effect of species ($F_{1, 71} = 0.35$, $P = 0.55$) or diet treatments ($F_{2, 71} = 1.07$, $P = 0.35$).

However, the time until pupation differed significantly between species ($F_{1, 70} = 61.4$,

$P < 0.0001$) and across diet treatments ($F_{2, 70} = 68.8$, $P < 0.0001$), with each pairwise

comparison between diet treatments significant at $P < 0.0001$. Since pupae cannot be

reliably sexed without dissection, I was unable to examine sex-specific differences in

time to pupation. Egg-to-adult development time, however, did show an effect of

sex, as well as species and diet quality (Table 6). Male *C. dalmanni* took longer to develop than females in all diet treatments ($P < 0.0001$ for each pairwise comparison), while this was only true for the high quality diet treatment in *C. quinqueguttata* (Fig. 9). In addition, *C. quinqueguttata* required more time than *C. dalmanni* to develop across all diet treatments, and diet quality was inversely related to egg-to-adult development time for both species (Fig. 9). The medium quality diet increased development time by 16% and 11% in female and male *C. dalmanni*, respectively, and 12% and 7% in female and male *C. quinqueguttata*. The low quality diet increased development time by 28% and 24% in female and male *C. dalmanni*, respectively, and 27% and 19% in female and male *C. quinqueguttata*.

Trait size and diet quality

Before examining effects of diet treatments on morphology, I first tested for overall differences between *C. dalmanni* and *C. quinqueguttata* using ANOVAs of each body part. The two species differed significantly for each test: eye-span ($F_{1, 442}$ = 6657.81, $P < 0.0001$), eye-bulb area ($F_{1, 442}$ = 3078.20, $P < 0.0001$), eye-stalk width ($F_{1, 442}$ = 3936.85, $P < 0.0001$), wing length ($F_{1, 442}$ = 401.11, $P < 0.0001$), and body length ($F_{1, 442}$ = 6657.81, $P < 0.0001$). Trait sizes were bigger on average in *C. quinqueguttata* than in *C. dalmanni* for each part measured except for eye-span, where the sexually dimorphic *C. dalmanni* clearly have longer eye-stalks overall (Fig. 10a). As food quality declined, both body size and absolute eye-span declined in male and female *C. dalmanni*. Body size and absolute eye-span remained unaffected by diet in both sexes of *C. quinqueguttata* (Table 7).

As each trait demonstrated a significant effect of body length ($P < 0.0001$ for each test), I tested the effect of diet on morphology separately for each species using ANCOVAs with body length as a covariate. Eye-span in *C. dalmanni* was significantly affected by diet quality, sex, and body length, whereas eye-span in *C. quinqueguttata* was only affected by body length (Table 8). Eye span increases with diet quality in *C. dalmanni* but is unaffected by diet in *C. quinqueguttata* (Table 8; Fig. 10a). Furthermore, when the sexes are tested separately within *C. dalmanni*, male eye-span is significantly affected by diet quality ($F_{2, 113} = 12.49$, $P < 0.0001$) while female eye-span is not ($F_{2, 162} = 2.61$, $P = 0.08$). ANCOVAs for eye-bulb area and eye-stalk width also reveal significant effects of diet for both species (Table 8). When the sexes are tested separately within each species, only males demonstrate an effect of diet quality on eye-bulb area (*C. dalmanni* males: $F_{2, 113} = 5.83$, $P = 0.004$, females: $F_{2, 162} = 1.58$, $P = 0.21$; *C. quinqueguttata* males: $F_{2, 70} = 4.17$, $P = 0.019$, females: $F_{2, 83} = 1.39$, $P = 0.25$; Fig. 10b). However, both sexes show an effect of diet on eye-stalk width in *C. dalmanni* (males: $F_{2, 113} = 25.67$, $P < 0.0001$; females: $F_{2, 162} = 17.07$, $P < 0.0001$) while only male eye-stalks responded in *C. quinqueguttata* (males: $F_{2, 70} = 6.19$, $P = 0.003$; females: $F_{2, 83} = 3.43$, $P = 0.06$; Fig. 10c). Wing length did not vary with diet quality in either species (Table 8; Fig. 10d).

Diet quality and external morphological trade-offs

In addition to identifying condition-sensitive traits, a primary goal of the diet manipulation experiment was to examine potential trade-offs between eye-span and associated external traits. As diet-induced changes in relative eye-span were only

79

present in *C. dalmanni* males, I tested for trade-offs between eye-span and other traits

in these flies by combining all individuals from all diet treatments. I calculated the

residuals from the least squares regression line of each trait on body length and

performed ANCOVAs on each trait with residual eye-span as a covariate. No

interactions between residual eye-span and diet quality were detected, indicating that

each trait responded similarly across treatments (eye-bulb area: $F_{2, 113} = 0.96$, $P =$

0.39; eye-stalk width: $F_{2, 113} = 2.14$, $P = 0.12$; wing length: $F_{2, 113} = 1.37$, $P = 0.26$).

Trade-offs are expected to result in a significant negative slope of the regression of

each residual trait on residual eye-span. However, significant positive slopes for each

trait indicate that no trade-offs between eye-span and these traits occurred (eye-bulb

area: $\beta = 0.034 \pm 0.012$, $t_{125} = 2.95$, $P = 0.004$; eye stalk width: $\beta = 0.007 \pm 0.003$,

$t_{125} = 2.84$, $P = 0.005$; wing length: $\beta = 0.267 \pm 0.033$, $t_{125} = 8.07$, $P < 0.0001$).

Diet quality, gonad size, and gonad trade-offs

An ANOVA of mean testis size revealed a significant effect of species ($F_{1, 74}$

$= 5.62$, $P = 0.020$) and diet treatment ($F_{2, 74} = 7.78$, $P = 0.0009$), as well as a

significant interaction between them ($F_{2, 74} = 18.42$, $P < 0.0001$), indicating that testes

in *C. dalmanni* and *C. quinqueguttata* responded differently to diet treatments. I

therefore analyzed each species separately using ANCOVAs with body size as a

covariate (Table 9). The results of these analyses show that testis size in *C. dalmanni*

is sensitive to larval diet quality, while testis size in *C. quinqueguttata* remains

unaffected by diet treatments (Fig. 11). Furthermore, pairwise comparisons indicate

that, in *C. dalmanni*, testis size was affected by both the medium and low quality diet

treatments, which did not differ from each other ($P = 0.37$), but were both

significantly different from the high quality diet treatment ($P < 0.0001$). Since both

testes and eye-span in *C. dalmanni* males are apparently sensitive to condition, I

tested for trade-offs between them in the manner described previously for other

morphological traits. Calculations of residual eye-span were restricted to those flies

for which testes measurements were also available. An ANCOVA on residual testis

size reveals a significant interaction between diet quality and residual eye-span ($F_{2, 44}$

$= 4.9$, $P = 0.01$), indicating that flies responded differently across diet treatments.

The regression of residual testis size on residual eye-span indicates that the most

stressful treatment results in a significant negative association between eye-span and

testes (Fig. 12).

In contrast to testis size, egg number and length in females was not affected

by larval diet treatments to either species (Fig. 13). An ANOVA of the number of

mature eggs showed no effect of species ($F_{1, 75} = 0.017$, $P = 0.90$) or diet treatment

($F_{2, 75} = 0.11$, $P = 0.89$; Fig. 13a). Egg length was also unaffected by diet treatment

($F_{2, 74} = 1.70$, $P = 0.19$) but differed significantly between species ($F_{1, 74} = 845.2$, $P <$

0.0001). Female *C. quinqueguttata* have longer eggs than female *C. dalmanni* (Fig.

13b). The number of mature eggs was unrelated to body length in either species (*C.*

dalmanni: $F_{1, 35} = 0.002$, $P = 0.97$; *C. quinqueguttata*: $F_{1, 34} = 1.80$, $P = 0.19$).

Similarly, egg length showed no relationship with body length in either species (*C.*

dalmanni: $F_{1, 34} = 0.006$, $P = 0.94$; *C. quinqueguttata*: $F_{1, 34} = 0.47$, $P = 0.50$).

Rearing *C. dalmanni* and *C. quinqueguttata* larvae in diet treatments varying in nutritional quality reveals that male eye-span is sensitive to condition in sexually dimorphic *C. dalmanni* but not in monomorphic *C. quinqueguttata*. Absolute eye-span declined with diet quality in female *C. dalmanni*, but this effect was abolished after controlling for body size. While eye-span was insensitive to condition in both sexes of *C. quinqueguttata*, other features of the eye-stalks (eye-bulb area and stalk width) were reduced in males reared in poorer diet treatments, similar to *C. dalmanni*. Testis size in *C. dalmanni* but not *C. quinqueguttata* was also reduced when larvae were reared in low quality food, while egg number and length remained unaffected in females of both species. No trade-offs were evident between eye-span and other external morphological traits, including eye-bulb area, eye-stalk width, and wing length. However, male *C. dalmanni* reared in the most stressful environment demonstrated a trade-off between eye-span and testes. These results are discussed in the context of resource allocation trade-offs between developing body parts.

Effects of diet on development time and body size

Body size is a critical life history trait affecting fitness (Roff 1992; Stearns 1992) that can change in response to environmental variation (Gotthard and Nylin 1995; Nylin and Gotthard 1998; Brakefield et al. 2003). For example, insects feeding on high-quality diets during development typically grow larger than those reared on poor quality diets (Chapman 1998). The adults of most holometabolous insects do not grow, and body size at eclosion is therefore determined prior to pupation. The

cues that insects use to regulate the timing of pupation are not well understood, but the attainment of a critical weight often plays a role (Nijhout 1994). In the tobacco hornworm, *Manduca sexta*, individuals deprived of food prior to reaching their critical weight will undergo supernumerary molts in order to continue feeding (Nijhout 1975). Furthermore, critical weight itself can vary according to diet quality (Davidowitz et al. 2003) and may influence the evolution of body size (D'Amico et al. 2001).

In *C. dalmanni* and *C. quinqueguttata*, reduction in larval diet quality caused an increase in the time until pupation, a result which is generally predicted by life history models (Gotthard and Nylin 1995). Compared to the high quality diet, the medium quality diet added approximately 2-4 days to the larval phase while the low quality diet added 4-6 days (Fig. 9). While critical weights were not measured in this study, the extension of the larval phase is consistent with a delay in pupation in order to attain additional weight through feeding (Nijhout 1994). However, adult body size declined in poorer diets in *C. dalmanni* only (Table 7). This result may indicate that lower diet quality reduced critical weights in *C. dalmanni* but not *C. quinqueguttata*. In all treatment groups, *C. quinqueguttata* required more days to complete development than *C. dalmanni*. In the absence of stress, males had longer egg-to-adult development times than females in both the dimorphic and monomorphic species (Fig. 9). Together, these results suggest that development time is likely not directly related to the production of exaggerated eye-stalks, as suggested by a previous study (Buschbeck et al. 2001).

Effects of diet on eye-span and associated structures

After controlling for body size, eye-span in male *C. dalmanni* alone demonstrated an effect of environmental stress (Fig. 10a). A non-sexual trait, wing length, was not affected by condition in either species (Fig. 10d). The heightened condition-dependence of exaggerated male eye-stalks described here agrees with patterns reported elsewhere. In *C. dalmanni*, experiments manipulating condition via larval density (David et al. 1998) and food quantity (Cotton et al. 2004a) have shown that male eye-span is more condition-sensitive than either female eye-span or other non-sexual traits. Both sexes demonstrate effects of condition on eye-span in *Diasemopsis aethiopica* (Knell et al. 1999), but the degree of sexual dimorphism in this species is less than other diopsids, with females having comparatively greater eye-span (Baker and Wilkinson 2001). In *Sphyracephala beccarri*, another species with a low degree of dimorphism (Baker and Wilkinson 2001), both male and female eye-span declined with greater food stress, but the overall response of eye-span to condition was not as great as in *C. dalmanni* (Cotton et al. 2004b). The results presented here further demonstrate a link between eye-span exaggeration and condition by revealing that eye-span in *C. quinqueguttata*, which is more closely related to *C. dalmanni* than *S. beccarri* (Wilkinson and Taper 1999; Baker and Wilkinson 2001) shows no response to nutritional stress. This supports the contention that the elaboration of eye-span within the Diopsidae has been associated with an increased sensitivity to environmental variation (Wilkinson and Taper 1999).

Collectively, the preceding results are consistent with the handicap hypothesis of sexual selection, which proposes that sexual traits such as exaggerated 'ornaments'

reveal the condition of their bearer, thereby allowing the opposite sex to choose high

quality mates (Zahavi 1975; Pomiankowski 1987; Iwasa et al. 1991; Iwasa and

Pomiankowski 1999).   A study of six species of stalk-eyed flies revealed over 20

times as much genetic variation for male eye-span in *C. dalmanni* as in *C.*

quinqueguttata, with evolutionary change in male eye-span genetic variance due

primarily to the relationship between eye-span and condition (Wilkinson and Taper

1999). Given that eye-span is used to assess males during both female choice

(Wilkinson and Reillo 1994) and male-male competition (Panhuis and Wilkinson

1999), the condition-dependence of this trait serves to provide additional information

over body size alone. Experimental evidence from other insects supports the

hypothesis that exaggerated sexual traits should demonstrate condition-dependence.

In carrion flies, *Prochyliza xanthostoma*, male sexual traits show a stronger negative

response to declining diet quality according to the degree of exaggeration

(Bonduriansky and Rowe 2005). The length of the exaggerated horns in male dung

beetles (*Onthophagus acuminatus*) is also strongly dependent on both food quantity

(Emlen 1994) and quality (Emlen 1997).

No evidence for external morphological trade-offs within species

Comparisons between species across diet treatments revealed that all traits

except eye-span are larger in *C. quinqueguttata* than in *C. dalmanni* (Fig. 10a-d).

Hence, an increase in the length of eye-stalks in the dimorphic *C. dalmanni* appears to

be associated with a reduction in the width of the stalk and the size of the eye, relative

to a monomorphic species. A phylogenetic analysis of 30 species within the family

Diopsidae established that monomorphism is the ancestral state for the family, with at least 4 independent evolutions of sexual dimorphism (Baker and Wilkinson 2001). Furthermore, head mass does not differ between *C. quinqueguttata* and *C. whitei*, a dimorphic congener morphologically similar to *C. dalmanni* (Swallow et al. 2000), or between *C. quinqueguttata* and *C. dalmanni* (J. Swallow, pers. comm.). The eye-stalk and eye both arise from cells within the eye-antennal imaginal disc (Buschbeck et al. 2001; Hurley et al. 2002), and similar head masses between dimorphic and monomorphic species suggest that the same amount of "raw materials" have been employed to build morphologically dissimilar heads. Collectively, these results suggest that the relative sizes of the eye and eye-stalk have been modified over an evolutionary timescale within the Diopsidae to exaggerate the eye-stalk from a monomorphic ancestor.

A reprogramming of the eye-antennal imaginal disc to create an elongated eye-stalk may be expected to increase the likelihood of within-disc trade-offs. However, no trade-offs between eye-span, eye-bulb area, or eye-stalk width were recorded within either *C. dalmanni* or *C. quinqueguttata* in any diet treatment. Nevertheless, the stress-induced reduction in eye-stalks and associated structures highlight several interesting patterns that may reflect differential resource allocation within and between species. First, in addition to the reduction in eye-span, male *C. dalmanni* demonstrated a decline in eye-bulb area and the width of the eye-stalk in poorer quality diets (Fig. 10b and c). The lack of trade-offs between these structures suggests that, as body size declined, the eye-stalks and associated structures declined by approximately equivalent amounts. Second, eye-span in both female *C. dalmanni*

and *C. quinqueguttata* remained unaffected by diet, but there was a significant effect of condition on eye-stalk width in *C. dalmanni* (Fig. 10c). This difference may reflect the relative length of the eye-stalks: although female eye-span is unexaggerated in both species, *C. dalmanni* have longer eye-span relative to *C. quinqueguttata* (Baker and Wilkinson 2001). Third, male *C. quinqueguttata*, which demonstrated no change in eye-span with declining diet quality, experienced a reduction in both eye-bulb size and eye-stalk width (Fig. 10b and c), revealing that condition may affect other parts of unexaggerated eye-stalks without influencing eye-span itself. This result was unexpected given that *C. quinqueguttata* are monomorphic, but it may indicate that male eye-stalk morphology, even when unexaggerated, displays greater condition dependence relative to other body parts, as suggested by a previous study (Cotton et al. 2004b).

Effects of diet on gonads and gonad trade-offs

The resources acquired during development may influence not only an organism's morphology, but also life history traits during adulthood, particularly reproduction (Roff 1992; Stearns 1992). In holometabolous insects, the exoskeleton is fixed soon after eclosion, but some internal structures, including the gut and gonad, have cells that continue to divide into adulthood (Nijhout 1994; Chapman 1998). The size of the gonads may therefore be influenced by resource acquisition during both larval development and adulthood. When food is limited, resources may be preferentially allocated to basic somatic maintenance, thereby reducing allocation to reproduction (Zera and Harshman 2001). For example, poor adult nutrition in male

87

Drosophila grimshawi induces a trade-off between somatic tissues and testis size (Droney 1998). In addition, the extent to which nutrition influences gonad development in one or more life stages may depend strongly on the ecology and natural history of the insect (Collins 1980). In *C. dalmanni*, which feed throughout adulthood, both males and females exhibit delayed maturity, with females reaching maturity around 3 weeks of age, and males at approximately 3 and a half weeks (Baker et al. 2003; Fry 2006). Adult dietary restriction increases the time required to reach sexual maturity and reduces testis length (Baker et al. 2003). The results presented here indicate that larval dietary restriction also reduces testis size in *C. dalmanni* but not *C. quinqueguttata* (Fig. 11).

The strong effect of larval diet on adult testis size in *C. dalmanni* is somewhat unexpected, given that adults were provided with high quality food *ad libitum* and the measurements were taken at 30 days of age. That the adults were unable to sufficiently compensate for poor larval diets via food intake supports the argument that testes and sperm are energetically expensive to produce (Pitnick et al. 1995; Pitnick 1996; Baker et al. 2003). Given that testes can continue to grow for several months after sexual maturity (Wilkinson et al. 2005), it is also possible that males were able to achieve some additional growth after 30 days. However, the relatively small growth rate experienced by older age classes makes true compensatory growth seem unlikely (Wilkinson et al. 2005). There was no effect of diet manipulation on testis size in *C. quinqueguttata*, suggesting that these flies were either able to compensate for poor larval diets, or that the testes require a smaller energetic investment than in *C. dalmanni*, or possibly both. The latter hypothesis is supported

by a comparison of testis size in the absence of stress, which reveals that *C. quinqueguttata* have significantly smaller testes (Fig. 11). In addition, *C. quinqueguttata* have lower rates of female remating and should therefore experience less sperm competition relative to *C. dalmanni* (Wilkinson et al. 1998; Panhuis and Wilkinson 1999), implying a reduced need to invest in testes.

Alternatively, the observed relationship between testes and eye-span in *C. dalmanni* may reflect a resource allocation trade-off. An examination of residual eye-span and testis sizes revealed that males in the most stressful treatment experienced a trade-off between testes and eye-span (Fig. 12). When nutrients were limiting, males allocated resources to eye-span at the expense of testes. Testis size may be largely determined during development, despite the fact that sexual maturity is delayed and adults have ample opportunities to gain additional resources. The trade-off between testes and eye-span supports the prediction that interactions between developing body parts play a role in determining their final sizes (Nijhout and Wheeler 1996). A study of horned beetles has also revealed a trade-off between primary and secondary sexual traits, where experimental ablation of the genital discs that give rise to male genitalia resulted in larger relative horn lengths (Moczek and Nijhout 2004).

In contrast to the effect of diet manipulation on gonad development in male *C. dalmanni*, females showed no change in egg size or egg number in either species (Fig. 13). This result is also in contrast to *D. melanogaster*, where larval diet limitation reduced female fecundity (Tu and Tatar 2003). Several explanations may account for these sex-specific differences. First, if larval survival is strongly dependent on egg size, as suggested in other Diptera (Collins 1980), egg size may be under stabilizing

selection and relatively insensitive to condition. Alternatively, if we presume that eye-stalk development is costly and is at least partially responsible for the reduction in gonad size in males, female gonad development may be relatively impervious to these costs due to their smaller eye-span. Another possibility is that egg maturation is more dependent on adult nutrition than testis development, allowing females the opportunity to compensate for poor larval diets through adult feeding.

Implications for resource allocation trade-offs

The results presented here demonstrate that larval nutrition can affect both adult morphology and gonad development. The effects of diet on testis size in *C. dalmanni* persist for at least 30 days into adulthood, demonstrating that the larval environment can exert effects across a developmental boundary. While this result is consistent with predictions for holometabolous insects (Nijhout and Emlen 1998; Emlen and Nijhout 2000; Emlen 2001; Emlen and Allen 2004), relatively few studies have demonstrated a relationship between larval nutrition and adult reproduction, and none of these examine males (Tu and Tatar 2003; Fischer et al. 2004; Bauerfeind and Fischer 2005; Boggs and Freeman 2005). The trade-off between eye-span and testes in *C. dalmanni* also emphasizes that antagonistic interactions during development are not necessarily restricted to body parts in close physical proximity (Moczek and Nijhout 2004; Tomkins et al. 2005; Fry 2006). Furthermore, evidence of trade-offs in *C. dalmanni* but not *C. quinqueguttata* supports the prediction that condition-dependent traits should be more likely to demonstrate trade-offs (Tomkins et al. 2004).

Given that testes are undoubtedly important for reproductive success, it is not obvious why trade-offs would manifest in the gonads. In *Drosophila*, approximately 50% of the germ cells originally formed are actually incorporated into the gonads, with the remaining cells undergoing programmed cell death (Sonnenblick 1950; Underwood et al. 1980; Coffman 2003). While the mechanism regulating this cell death is largely unknown, recent studies suggest that a lipid signaling system may be involved (Coffman 2003). Interestingly, lipid signaling has also been proposed as a mechanism underlying the action of juvenile hormone (JH) (Wheeler and Nijhout 2003), a developmental hormone that has been implicated in a variety of morphological and life-history trade-offs in flies, crickets, and beetles (Dingle and Winchell 1997; Nijhout and Emlen 1998; Zera et al. 1998a, 1998b; Emlen and Nijhout 1999; Cisper et al. 2000; Salmon et al. 2001; Tatar et al. 2001; Flatt and Kawecki 2004; Flatt et al. 2005). As JH induces a trade-off between testis size and eye-span in *C. dalmanni* (Fry 2006), the similar diet-induced trade-off reported here may thus arise via a common signaling pathway operating during ontogeny. Alternatively, reductions in testis size may simply result from reduced abdomen size. Males of the morphologically similar congener *C. whitei* have larger thoraxes and smaller abdomens for their body size relative to female *C. whitei* or either sex of *C. quinqueguttata* (Swallow et al. 2000). The authors suggest that the relative sizes of the thorax and abdomen indicate that flight muscle and testis size may trade off (Swallow et al. 2000).

Recent evidence from *C. dalmanni* also suggests that accessory gland size may be equally or more important for reproductive success than testis size (Baker et

al. 2003; Pomiankowski et al. 2005; Rogers et al. 2005). The accessory glands produce seminal fluid as well as proteins that form the spermatophore into which sperm are packaged during copulation (Kotrba 1996). Seminal fluid strongly influences the outcome of sperm competition in *Cyrtodiopsis whitei* (Fry and Wilkinson 2004), and both *C. whitei* and *C. dalmanni* pass remarkably few sperm per copulation (34 and 66 on average, respectively) (Fry and Wilkinson 2004; Fry 2006). The effect of reductions in testis size on reproductive fitness therefore remains unclear. Accessory glands were not measured in this study, but it would be informative to note what effect, if any, larval nutrition has on adult accessory gland size.

While the nature and magnitude of trade-offs have become increasingly well documented in insects, the mechanisms that mediate these trade-offs are less clear. The pleiotropic nature of hormones and hormone metabolism has led researchers to suggest that endocrine regulation underlies trade-offs (Ketterson and Nolan 1992; Finch and Rose 1995; Dingle and Winchell 1997; Sinervo and Svensson 1998; Flatt et al. 2005). That manipulations of JH and nutrition can often induce similar responses underscores the potential relationship between resources and JH biosynthesis and signaling (Emlen and Nijhout 2000; Emlen and Allen 2004). In *C. dalmanni*, application of the JH agonist methoprene during larval development causes small males to produce longer eye-stalks relative to their body length (Fry 2006). In addition, methoprene treatment results in consistently smaller testes for at least 30 days into adulthood (Fry 2006). Hence, both JH and resource limitation can induce trade-offs between eye-span and testes, suggesting that the effects of nutrition on

morphology and reproduction may be mediated by JH. While the molecular

mechanism of JH-mediated trade-offs is still unclear (Flatt et al. 2005), evidence from

Drosophila indicates that *methoprene-tolerant* (*Met*), a gene that codes for a putative

juvenile hormone receptor, has pleiotropic effects on life history traits (Flatt and

Kawecki 2004) and reproduction (Wilson et al. 2003). Future work will be required

to disentangle the effects of nutrition and JH, but current evidence suggests that the

study of endocrine loci may reveal causal links between resource allocation and trade-

offs.

TABLE 6. The results of an ANOVA on egg-to-adult development time.

Source of variation	df	*F*	*P*
Species	1	319.6	<0.0001
Diet quality	2	171.6	<0.0001
Sex	1	8.84	0.003
Species x Diet quality	2	0.62	0.54
Species x sex	1	8.74	0.003
Diet quality x sex	2	2.02	0.13
Error	395		

TABLE 7. Absolute (mm) trait sizes (mean $\pm$ SE) of flies developing in each diet treatment. Body length was affected by diet in both male and female *C. dalmanni* ($F_{2, 116} = 15.25$, $P < 0.0001$ and $F_{2, 165} = 10.58$, $P < 0.0001$, respectively) but was unrelated to diet in either sex of *C. quinqueguttata* ($P \geq 0.77$). Similarly, absolute eye-span was affected by diet in male and female *C. dalmanni* ($F_{2, 116} = 31.81$, $P < 0.0001$ and $F_{2, 165} = 13.35$, $P < 0.0001$, respectively) but in neither sex of *C. quinqueguttata* ($P \geq 0.66$).

Species	Diet quality	Body length	Eye-span
C. dalmanni			
	low	6.63 ± 0.03	7.72 ± 0.07
Male	medium	6.83 ± 0.04	8.05 ± 0.05
	high	6.96 ± 0.04	8.38 ± 0.05
	low	6.58 ± 0.04	5.59 ± 0.04
Female	medium	6.64 ± 0.05	5.68 ± 0.04
	high	6.78 ± 0.03	5.82 ± 0.03
C. quinqueguttata			
	low	7.38 ± 0.04	4.47 ± 0.02
Male	medium	7.39 ± 0.05	4.46 ± 0.03
	high	7.42 ± 0.07	4.50 ± 0.04
	low	7.67 ± 0.04	4.56 ± 0.03
Female	medium	7.66 ± 0.07	4.53 ± 0.02
	high	7.70 ± 0.06	4.54 ± 0.03

TABLE 8. F-ratios from ANCOVAs on eye-span, eye-bulb area, eye-stalk width, and wing length with body length as a covariate. Significant *P*-values are indicated at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.0001$ (***).

Source of variation	df	eye-span	eye-bulb	eye-stalk	wing
C. dalmanni					
Diet quality	2	16.2***	6.3**	39.2***	3.5
Sex	1	6327.6***	3.5	65.9***	19.6***
Body length	1	260.2***	62.6***	23.2***	188.6***
Diet quality x sex	2	5.7**	1.2	3.4	0.3
Body length x diet quality	2	3.9*	1.6	0.9	3.4
Body length x sex	1	17.5***	0.05	11.6**	1.0
Error	277				
C. quinqueguttata					
Diet quality	2	0.2	3.8*	9.1**	2.9
Sex	1	1.7	6.6*	7.7**	6.4*
Body length	1	51.67***	16.3***	0.2	26.3***
Diet quality x sex	2	1.7	3.6*	1.6	3.6*
Body length x diet quality	2	2.0	4.7*	3.2*	1.9
Body length x sex	1	4.9*	0.9	2.2	0.9
Error	155				

TABLE 9. The results of an ANCOVA on mean testis size with body length as a

covariate.

| Source of variation	df	*F*	*P*
C. dalmanni			
Diet quality	2	31.47	<0.0001
Body length	1	0.40	0.53
Quality x body length	2	0.86	0.43
Error	44		
C. quinqueguttata			
Diet quality	2	1.06	0.36
Body length	1	0.82	0.37
Quality x body length	2	0.03	0.97
Error	24		

FIG. 9. Mean egg-to-adult development time of flies reared in diets that vary in quality. Error bars indicate ± SE and bar colors indicate males (gray) and females (white). Dashed lines indicate mean time until pupation.

FIG. 10. Least squares means of morphological traits of adults emerging from larval diet treatments: a) eye-span (mm), b) eye-bulb area (mm^2), c) eye-stalk width (mm), and d) wing length (mm). Error bars indicate ± SE and bar colors indicate males (gray) and females (white). L = low, M = medium, H = high.

FIG. 11. Least squares mean (± SE) testis area of adult males emerging from larval diet treatments (black bars = *C. dalmanni*; white bars = *C. quinqueguttata*).

FIG. 12. Scatterplot and least squares regression lines for residual eye-span and residual testis size of 30-day old adult males emerging from larval diet treatments. Symbols and regression line patterns indicate diet treatment type: high quality = open squares, solid line (β = 0.17 ± 0.10, t_{15} = 1.65, *P* = 0.12); medium quality = filled squares, dotted line (β = 0.23 ± 0.15, t_{14} = 1.55, *P* = 0.14); low quality = open circles, broken line (β = - 0.27 ± 0.13, t_{15} = 2.79, *P* = 0.03).
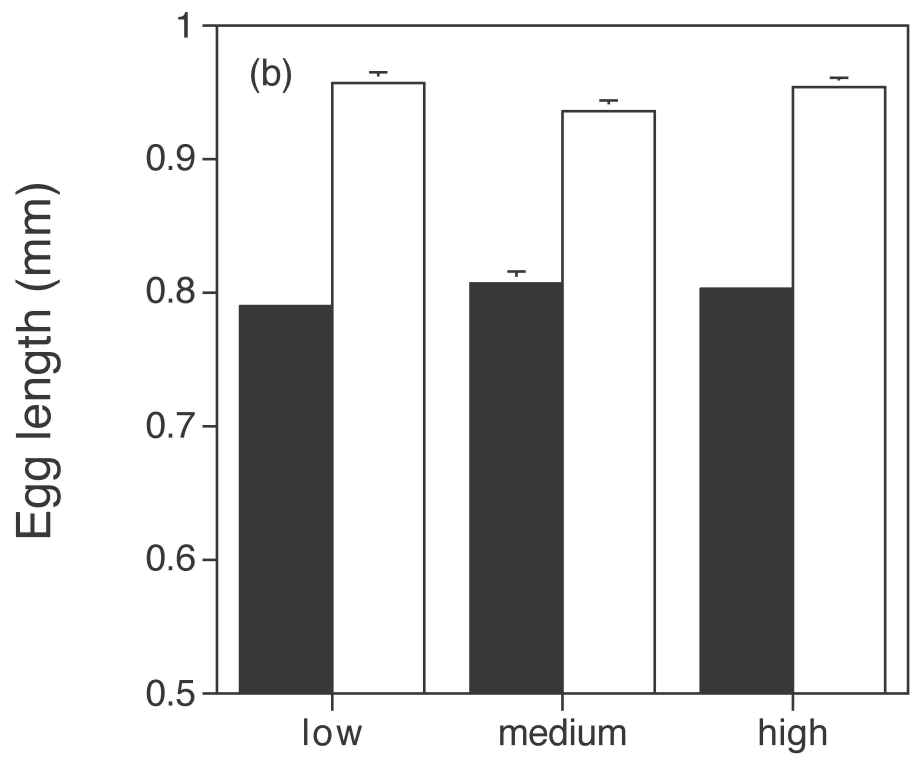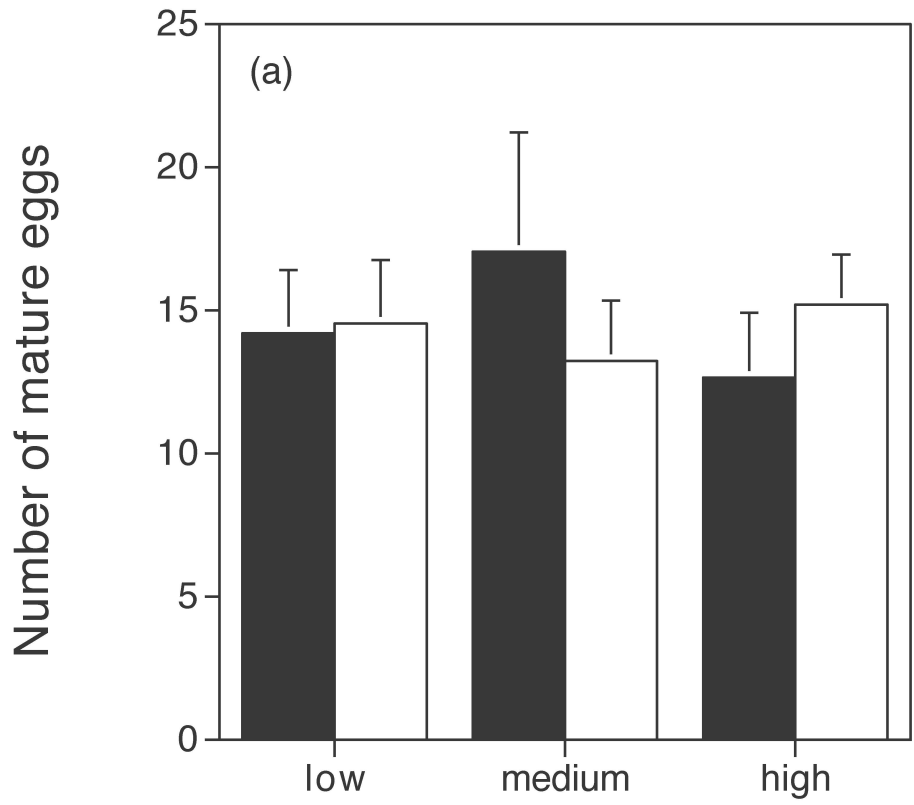
FIG. 13. Least squares mean (± SE) a) number of mature eggs and b) egg length in adult females emerging from larval diet treatments (black bars = *C. dalmanni*; white bars = *C. quinqueguttata*).
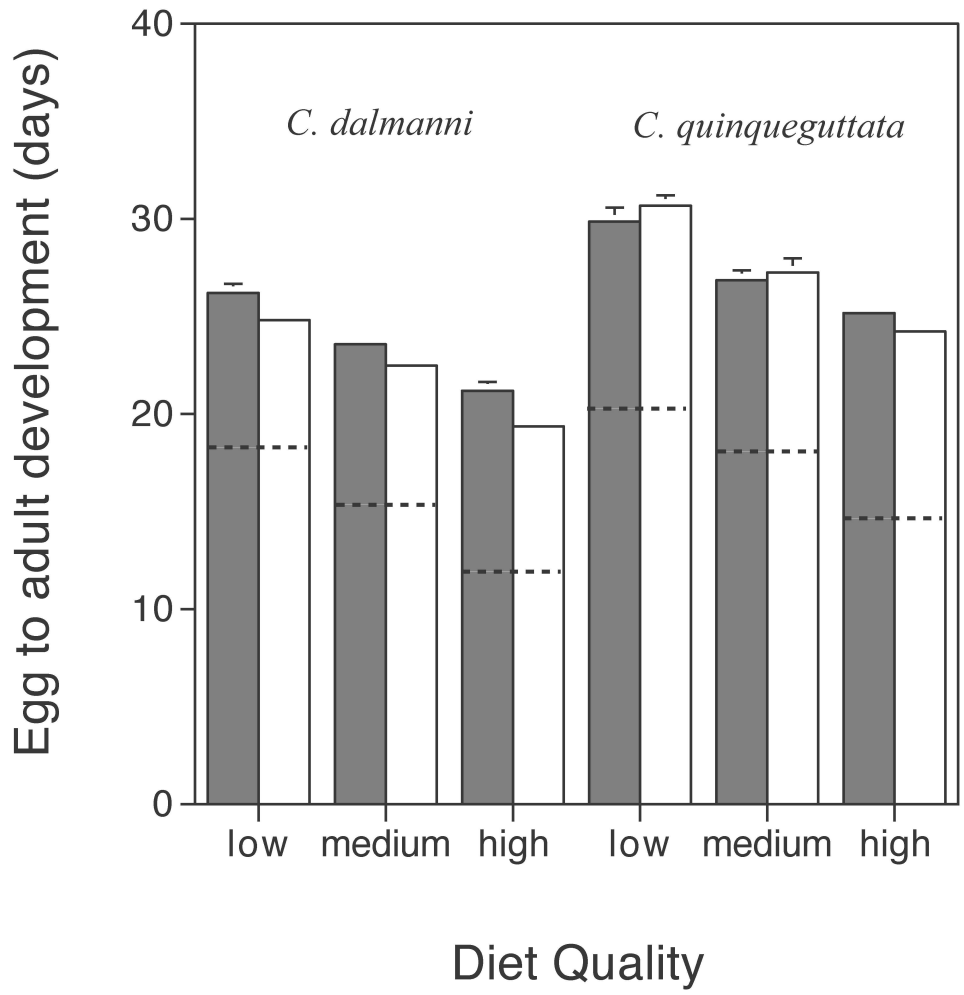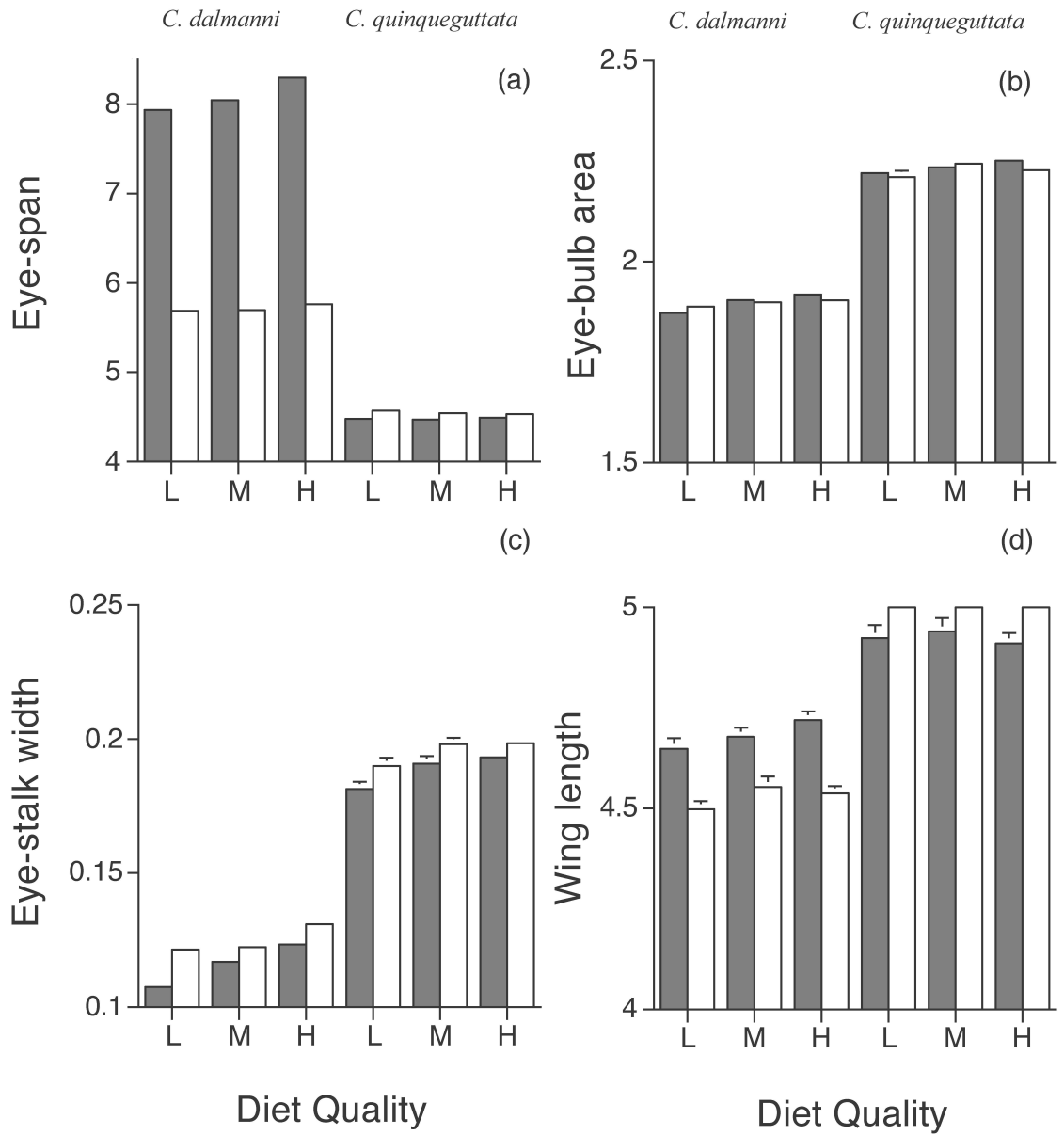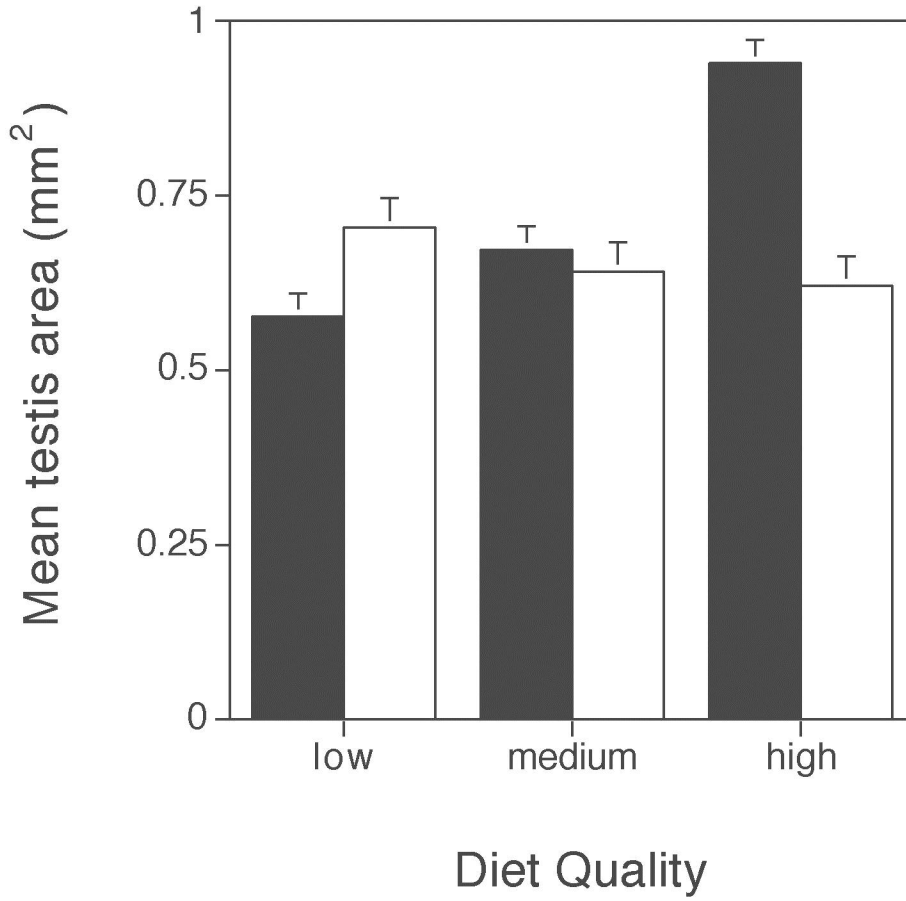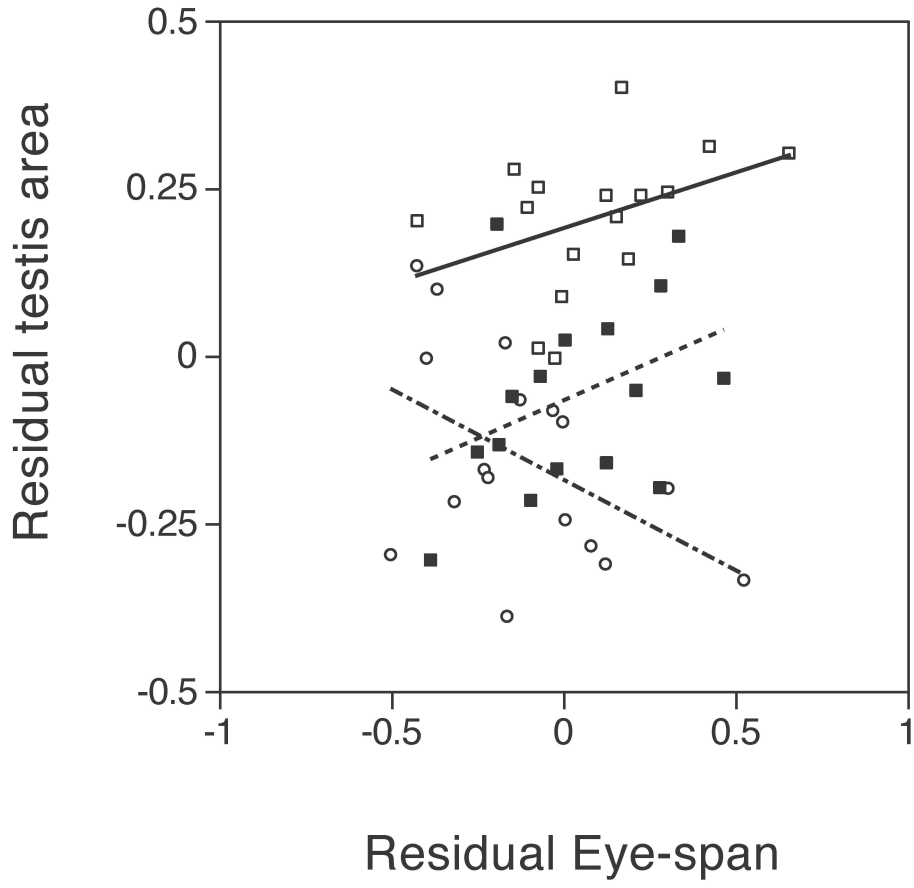
LITERATURE CITED

Andersson, M. 1994. *Sexual selection*. Princeton, New Jersey: Princeton University
Press.

Arnold, S. J. 1992. Constraints on phenotypic evolution. *Am. Nat.* 140, S85-S107.

Baker, R. H. and Wilkinson, G. S. 2001. Phylogenetic analysis of sexual dimorphism
and eye-span allometry in stalk-eyed flies (Diopsidae). *Evolution* 55, 1373-
1385.

Baker, R. H., Denniff, M., Futerman, P., Fowler, K., Pomiankowski, A. and
Chapman, T. 2003. Accessory gland size influences time to sexual maturity
and mating frequency in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. *Behav.
Ecol.* 14, 607-611.

Barnes, A. I. and Partridge, L. 2003. Costing reproduction. *Anim. Behav.* 66, 199-204.

Bauerfeind, S. S. and Fischer, K. 2005. Effects of food stress and density in different
life stages on reproduction in a butterfly. *Oikos* 111, 514-524.

Bessa, J. and Casares, F. 2001. The regulation of homothorax and the control of eye-
head proportion in *Drosophila*. *Int. J. Dev. Biol.* 45, S135-S136.

Boggs, C. L. and Freeman, K. D. 2005. Larval food limitation in butterflies: Effects
on adult resource allocation and fitness. *Oecologia* 144, 353-361.

Bollenbacher, W. E. 1988. The interendocrine regulation of larval pupal development
in the tobacco hornworm, *Manduca sexta* - a model. *J. Insect Physiol.* 34,
941-947.

Bonduriansky, R. and Day, T. 2003. The evolution of static allometry in sexually
selected traits. *Evolution* 57, 2450-2458.

Bonduriansky, R. and Rowe, L. 2005. Sexual selection, genetic architecture, and the condition dependence of body shape in the sexually dimorphic fly *Prochyliza xanthostoma* (Piophilidae). *Evolution* 59, 138-151.

Boots, M. and Begon, M. 1994. Resource limitation and the lethal and sublethal effects of a viral pathogen in the Indian meal moth, *Plodia interpunctella*. *Ecol. Entomol.* 19, 319-326.

Bouchard, B. L. and Wilson, T. G. 1987. Effects of sublethal doses of methoprene on reproduction and longevity of *Drosophila melanogaster* (Diptera, Drosophilidae). *J. Econ. Entomol.* 80, 317-321.

Brakefield, P. M., French, V. and Zwaan, B. J. 2003. Development and the genetics of evolutionary change within insect species. *Annu. Rev. Ecol. Evol. Syst.* 34, 633-660.

Burkhardt, D., Delamotte, I. and Lunau, K. 1994. Signaling fitness: Larger males sire more offspring - studies of the stalk-eyed fly *Cyrtodiopsis white*i (Diopsidae, Diptera). *J. Comp. Physiol.* A 174, 61-64.

Buschbeck, E. K. and Hoy, R. R. 1998. Visual system of the stalk-eyed fly, *Cyrtodiopsis quinqueguttata* (Diopsidae, Diptera): An anatomical investigation of unusual eyes. *J. Neurobiol.* 37, 449-468.

Buschbeck, E. K., Roosevelt, J. L. and Hoy, R. R. 2001. Eye stalks or no eye stalks: A structural comparison of pupal development in the stalk eyed fly *Cyrtodiopsis* and in *Drosophila*. *J. Comp. Neurol.* 433, 486-498.

Chapman, R. F. 1998. *The insects: Structure and function*. Cambridge: Cambridge University Press.

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

Cheverud, J. M. 1984. Quantitative genetics and developmental constraints on evolution by selection. *J. Theor. Biol.* 110, 155-171.

Cisper, G., Zera, A. J. and Borst, D. W. 2000. Juvenile hormone titer and morph-specific reproduction in the wing-polymorphic cricket, *Gryllus firmus*. *J. Insect Physiol.* 46, 585-596.

Clutton-Brock, T. H., Guinness, F. E. and Albon, S. D. 1982. *Red deer: Behavior and ecology of two sexes*. Chicago: University of Chicago Press.

Coffman, C. R. 2003. Cell migration and programmed cell death of *Drosophila* germ cells. *Ann. N.Y. Acad. Sci.* 995, 117-126.

Cock, A. G. 1966. Genetical aspects of metrical growth and form in animals. *Quart. Rev. Biol.* 41, 131-190.

Collins, N. C. 1980. Developmental responses to food limitation as indicators of environmental conditions for *Ephydra cinerea* Jones (Diptera). *Ecology* 61, 650-661.

Cotton, S., Fowler, K. and Pomiankowski, A. 2004a. Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera : Diopsidae). *Evolution* 58, 1038-1046.

Cotton, S., Fowler, K. and Pomiankowski, A. 2004b. Heightened condition dependence is not a general feature of male eyespan in stalk-eyed flies (Diptera : Diopsidae). *J. Evol. Biol.* 17, 1310-1316.

Cowley, D. E. and Atchley, W. R. 1990. Development and quantitative genetics of correlation structure among body parts of *Drosophila melanogaster*. *Am. Nat.* 135, 242-268.

D'Amico, L. J., Davidowitz, G. and Nijhout, H. F. 2001. The developmental and physiological basis of body size evolution in an insect. *Proc. R. Soc. Lond.* B 268, 1589-1593.

Darwin, C. 1859. *On the origin of species*. London: Murray.

David, P., Hingle, A., Greig, D., Rutherford, A., Pomiankowski, A. and Fowler, K. 1998. Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. *Proc. R. Soc. Lond.* B 265, 2211-2216.

David, P., Bjorksten, T., Fowler, K. and Pomiankowski, A. 2000. Condition-dependent signalling of genetic variation in stalk-eyes flies. *Nature* 406, 186-188.

Davidowitz, G., D'Amico, L. J. and Nijhout, H. F. 2003. Critical weight in the development of insect body size. *Evol. Dev* 5, 188 -197.

deWilde, J. 1976. Juvenile hormone and caste differentiation in the honey bee (*Apis mellifera* L.). In *Phase and Caste Determination in Insects: Endocrine Aspects* (ed. M. Luscher). New York: Pergamon. pp. 5-20.

deWilde, J. and Beetsma, J. 1982. The physiology of caste development in social insects. *Adv. Insect Physiol.* 16, 167-246.

Dingle, H. and Winchell, R. 1997. Juvenile hormone as a mediator of plasticity in insect life histories. *Arch. Insect Biochem. Physiol.* 35, 359-373.

Droney, D. C. 1998. The influence of the nutritional content of the adult male diet on

testis mass, body condition, and courtship vigour in Hawaiian *Drosophila*.

Funct. Ecol. 12, 920-928.

Emlen, D. J. 1994. Environmental control of horn length dimorphism in the beetle

Onthophagus acuminatus (Coleoptera, Scarabaeidae). *Proc. R. Soc. Lond.* B

256, 131-136.

Emlen, D. J. 1997. Diet alters male horn allometry in the beetle *Onthophagus

acuminatus* (Coleoptera: Scarabaeidae). *Proc. R. Soc. Lond.* B 264, 567-574.

Emlen, D. J. 2000. Integrating development with evolution: A case study with beetle

horns. *Bioscience* 50, 403-418.

Emlen, D. J. 2001. Costs and the diversification of exaggerated animal structures.

Science 291, 1534-1536.

Emlen, D. J. and Nijhout, H. F. 1999. Hormonal control of male horn length

dimorphism in the dung beetle *Onthophagus taurus* (Coleoptera:

Scarabaeidae). *J. Insect Physiol.* 45, 45-53.

Emlen, D. J. and Nijhout, H. F. 2000. The development and evolution of exaggerated

morphologies in insects. *Annu. Rev. Entomol.* 45, 661-708.

Emlen, D. J. and Allen, C. E. 2004. Genotype to phenotype: Physiological control of

trait size and scaling in insects. *Integr. Comp. Biol.* 43, 617-634.

Falconer, D. S. 1960. *Introduction to quantitative genetics*. New York: Ronald Press

Co.

Finch, C. E. and Rose, M. R. 1995. Hormones and the physiological architecture of

life history evolution. *Quart. Rev. Biol.* 70, 1-52.

Fischer, K., O'Brien, D. M. and Boggs, C. L. 2004. Allocation of larval and adult resources to reproduction in a fruit-feeding butterfly. *Funct*. *Ecol*. 18, 656-663.

Flatt, T. and Kawecki, T. J. 2004. Pleiotropic effects of *methoprene-tolerant* (*Met*), a gene involved in juvenile hormone metabolism, on life history traits in *Drosophila melanogaster*. *Genetica* 122, 141-160.

Flatt, T., Tu, M. P. and Tatar, M. 2005. Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. *Bioessays* 27, 999-1010.

Fry, C. L. 2006. Juvenile hormone mediates a trade-off between primary and secondary sexual traits in stalk-eyed flies. *Evol*. *Dev* 8, 191-201.

Fry, C. L. and Wilkinson, G. S. 2004. Sperm survival in female stalk-eyed flies depends on seminal fluid and meiotic drive. *Evolution* 58, 1622-1626.

Fuller, R. C., Baer, C. F. and Travis, J. 2005. How and when selection experiments might actually be useful. *Integ*. *Comp*. *Biol*. 45, 391-404.

Gotthard, K. and Nylin, S. 1995. Adaptive plasticity and plasticity as an adaptation - a selective review of plasticity in animal morphology and life-history. *Oikos* 74, 3-17.

Houle, D. 1991. Genetic covariance of fitness correlates - what genetic correlations are made of and why it matters. *Evolution* 45, 630-648.

Hurley, I., Fowler, K., Pomiankowski, A. and Smith, H. 2001. Conservation of the expression of *dll*, *en*, and *wg* in the eye-antennal imaginal disc of stalk-eyed flies. *Evol*. *Dev*. 3, 408-414.

Hurley, I., Pomiankowski, A., Fowler, K. and Smith, H. 2002. Fate map of the eye-antennal imaginal disc in the stalk-eyed fly *Cyrtodiopsis dalmanni*. *Dev. Gen. Evol.* 212, 38-42.

Huxley, J. S. 1932. *Problems of relative growth* London: Methuen and Co., Ltd.

Iwasa, Y., Pomiankowski, A. and Nee, S. 1991. The evolution of costly mate preferences. 2. The handicap principle. *Evolution* 45, 1431-1442.

Iwasa, Y. and Pomiankowski, A. 1999. Good parent and good genes models of handicap evolution. *J. Theor. Biol.* 200, 97-109.

Johnston, L. A. and Gallant, P. 2002. Control of growth and organ size in *Drosophila*. *Bioessays* 24, 54-64.

Kawano, K. 1995. Horn and wing allometry and male dimorphism in giant rhinoceros beetles (Coleoptera, Scarabaeidae) of tropical Asia and America. *Ann. Entomol. Soc. Am.* 88, 92-99.

Kawano, K. 1997. Cost of evolving exaggerated mandibles in stag beetles (Coleoptera: Lucanidae). *Ann. Entomol. Soc. Am.* 90, 453-461.

Kawano, K. 2004. Developmental stability and adaptive variability of male genitalia in sexually dimorphic beetles. *Am. Nat.* 163, 1-15.

Ketterson, E. D. and Nolan, V. 1992. Hormones and life histories - an integrative approach. *Am. Nat.* 140, S33-S62.

Klingenberg, C. P. and Nijhout, H. F. 1998. Competition among growing organs and developmental control of morphological asymmetry. *Proc. R. Soc. Lond.* B 265, 1135-1139.

Knell, R. J., Fruhauf, N. and Norris, K. A. 1999. Conditional expression of a sexually

    selected trait in the stalk-eyed fly *Diasemopsis aethiopica*. *Ecol*. *Entomol*. 24,

    323-328.

Knell, R. J., Pomfret, J. C., and Tomkins, J. L. 2004. The limits of elaboration: curved

    allometries reveal the constraints on mandible size in stag beetles. *Proc*. *R*.

    Soc. *Lond*. B 271, 523-528.

Kotrba, M. 1991. Das Reproduktionssystem von *Cyrtodiopsis whitei* Curran 1936

    (Diptera: Diopsidae) unter besonderer Berücksichtigung der inneren

    weiblichen Geschlechtsorgane (PhD thesis). Regensberg: University of

    Regensberg.

Kotrba, M. 1996. Sperm transfer by spermatophore in Diptera: New results from the

    Diopsidae. *Zool*. *J*. *Linn*. *Soc*. 117, 305-323.

Leroi, A. M. 2001. Molecular signals versus the *Loi de Balancement*. *TREE* 16, 24-

    29.

Lorch, P. D., Wilkinson, G. S. and Reillo, P. R. 1993. Copulation duration and sperm

    precedence in the stalk-eyed fly *Cyrtodiopsis whitei* (Diptera, Diopsidae).

    Behav. *Ecol*. *Sociobiol*. 32, 303-311.

Manning, A. 1966. Corpus allatum and sexual receptivity in female *Drosophila*

    melanogaster. *Nature* 211, 1321-1322.

Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B.,

    Lande, R., Raup, D. and Wolpert, L. 1985. Developmental constraints and

    evolution. *Quart*. *Rev*. *Biol*. 60, 265-287.

Moczek, A. P. and Nijhout, H. F. 2002. Developmental mechanisms of threshold

    evolution in a polyphenic beetle. *Evol*. *Dev*4, 252 -264.

Moczek, A. P. and Nijhout, H. F. 2004. Trade-offs during the development of primary

    and secondary sexual traits in a horned beetle. *Am. Nat.* 163, 184-191.

Nijhout, H. F. 1994. *Insect hormones*. Princeton, New Jersey: Princeton University

    Press.

Nijhout, H. F. 1975. Threshold size for metamorphosis in tobacco hornworm,

    Manduca sexta. *Biol*. *Bull*. 149, 214-225.

Nijhout, H. F. 2003. Development and evolution of adaptive polyphenisms. *Evol*.

    Dev. 5, 9-18.

Nijhout, H. F. and Wheeler, D. E. 1996. Growth models of complex allometries in

    holometabolous insects. *Am*. *Nat*. 148, 40-56.

Nijhout, H. F. and Emlen, D. J. 1998. Competition among body parts in the

    development and evolution of insect morphology. *Proc*. *Natl*. *Acad*. *Sci*. USA

    95, 3685-3689.

Norry, F. M., Vilardi, J. C. and Hasson, E. 1997. Genetic and phenotypic correlations

    among size-related traits, and heritability variation between body parts in

    Drosophila buzzatii. *Genetica* 101, 131-139.

Norry, F. M., Vilardi, J. C. and Hasson, E. 2000. Negative genetic correlation

    between traits of the *Drosophila* head, and interspecific divergence in head

    shape. *Heredity* 85, 177-183.

Nunney, L. 1996. The response to selection for fast larval development in *Drosophila melanogaster* and its effect on adult weight: An example of a fitness trade-off. *Evolution* 50, 1193-1204.

Nylin, S. and Gotthard, K. 1998. Plasticity in life-history traits. *Ann. Rev. Entomol.* 43, 63-83.

Ono, S. 1982. Effect of juvenile hormone on caste determination in the ant, *Pheidole fervida* Smith (Hymenoptera, Formicidae). *Appl. Entomol. Zool.* 17, 1-7.

Osorio, S., Piulachs, M. D., Belles, X. 1998. Feeding and activation of corpora allata in the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae). *J. Insect Physiol.* 44, 31-38.

Panhuis, T. M. and Wilkinson, G. S. 1999. Exaggerated male eye span influences contest outcome in stalk-eyed flies (Diopsidae). *Behav. Ecol. Sociobiol.* 46, 221-227.

Partridge, L. and Sibly, R. 1991. Constraints in the evolution of life histories. *Phil. Tran. R. Soc. Lond.* B 332, 3-13.

Petrie, M. 1992. Are all secondary sexual display structures positively allometric and, if so, why? *Anim. Behav.* 43, 173-175.

Pitnick, S. 1996. Investment in testes and the cost of making long sperm in *Drosophila. Am. Nat.* 148, 57-80.

Pitnick, S., Markow, T. A. and Spicer, G. S. 1995. Delayed male maturity is a cost of producing large sperm in *Drosophila. Proc. Natl. Acad. Sci.* USA 92, 10614 - 10618.

Pitnick, S. and Miller, G. T. 2000. Correlated response in reproductive and life

  history traits to selection on testis length in *Drosophila hydei*. *Heredity* 84,

  416-426.

Pomiankowski, A. 1987. Sexual selection - the handicap principle does work

  sometimes. *Proc. R. Soc. Lond.* B 231, 123-145.

Pomiankowski, A., Denniff, M., Fowler, K. and Chapman, T. 2005. The costs and

  benefits of high early mating rates in male stalk-eyed flies, *Cyrtodiopsis*

  dalmanni. *J. Insect Physiol*. 51, 1165-1171.

Postlethwait, J. H. 1974. Juvenile hormone and adult development of *Drosophila*.

  Biol. Bull. 147, 119-135.

Prasad, N. G., Shakarad, M., Anitha, D., Rajamani, M. and Joshi, A. 2001. Correlated

  responses to selection for faster development and early reproduction in

  Drosophila: The evolution of larval traits. *Evolution* 55, 1363-1372.

Restifo, L. L. and Wilson, T. G. 1998. A juvenile hormone agonist reveals distinct

  developmental pathways mediated by ecdysone-inducible Broad Complex

  transcription factors. *Dev. Genet*. 22, 141-159.

Riddiford, L. M. 1994. Cellular and molecular actions of juvenile hormone. I. General

  considerations and premetamorphic action. *Adv. Insect Physiol*. 24, 213-274.

Riddiford, L. M. and Ashburner, M. 1991. Effects of juvenile hormone mimics on

  larval development and metamorphosis of *Drosophila melanogaster Gen.*

  Comp. Endocrinol. 82, 172-183.

Riska, B. 1986. Some models for development, growth, and morphometric

  correlation. *Evolution* 40, 1303-1311.

Roff, D. A. 1992. *The evolution of life histories. Theory and analysis*. New York: Chapman and Hall.

Roff, D. A. 1997. *Evolutionary quantitative genetics*. New York: Chapman and Hall.

Rogers, D. W., Chapman, T., Fowler, K. and Pomiankowski, A. 2005. Mating-induced reduction in accessory reproductive organ size in the stalk-eyed fly *Cyrtodiopsis dalmanni. BMC Evol. Biol*. 5:37.

Rountree, D. B. and Bollenbacher, W. E. 1986. The release of the prothoracicotropic hormone in the tobacco hornworm, *Manduca sexta*, is controlled intrinsically by juvenile hormone. *J. Exp. Biol*. 120, 41-58.

Rowe, L. and Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond*. B 263, 1415-1421.

Salmon, A. B., Marx, D. B., and Harshman, L. G. 2001. A cost of reproduction in *Drosophila melanogaster*: stress susceptibility. *Evolution* 55, 1600-1608.

Saunders, D. S., Richard, D. S., Applebaum, S. W., Ma, M. and Gilbert, L. I. 1990. Photoperiodic diapause in *Drosophila melanogaster* involves a block to the juvenile hormone regulation of ovarian maturation. *Gen. Comp. Endocrinol*. 79, 174-184.

Simmons, L. W. and Tomkins, J. L. 1996. Sexual selection and the allometry of earwig forceps. *Evol. Ecol*. 10, 97-104.

Sinervo, B. and Basolo, A. L. 1996. Testing adaptation using phenotypic manipulations. In *Adaptation* (ed. M. Rose and G. Lauder), pp. 149-185. San Diego, CA: Academic.

Sinervo, B. and Svensson, E. 1998. Mechanistic and selective causes of life history trade-offs and plasticity. *Oikos* 83, 432-442.

Sonnenblick, B. P. 1950. The early embryology of *Drosophila melanogaster* In *Biology of Drosophila*, pp. 62-167. New York: Wiley.

Stearns, S. C. 1992. *The evolution of life histories*. Oxford: Oxford University Press.

Stearns, S., deJong, G. and Newman, B. 1991 The effects of phenotypic plasticity on genetic correlations. *TREE* 6, 122-126.

Stern, D. L. and Emlen, D. J. 1999. The developmental basis for allometry in insects. *Development* 126, 1091-1101.

Stevens, D. J., Hansell, M. H., Freel, J. A. and Monaghan, P. 1999. Developmental trade-offs in caddis flies: increased investment in larval defense alters adult resource allocation. *Proc. R. Soc. Lond.* B 266, 1049-1054.

Swallow, J. G., Wilkinson, G. S. and Marden, J. H. 2000. Aerial performance of stalk-eyed flies that differ in eye span. *J. Comp. Physiol.* B *Biochem. Syst. Environ. Physiol.* 170, 481-487.

Swallow, J. G., Wallace, L. E., Christianson, S. J., Johns, P. M., and Wilkinson, G. S. 2005. Genetic divergence does not predict change in ornament expression among populations of stalk-eyed flies. *Mol. Ecol.* 14, 3787-3800.

Tatar, M., Chien, S. A., and Priest, N. K. 2001. Negligible senescence during reproductive dormancy in *Drosophila melanogaster Am . Nat.* 158, 248-258.

Teal, P. E. A., Gomez Simuta, Y. and Proveaux, A. T. 2000. Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. *Proc. Natl. Acad. Sci. USA* 97, 3708- 3712.

Thompson, D. A. 1917. *On growth and form. Abridged ed. J. T. Bonner (ed.)*:

    Cambridge University Press.

Tobe, S. S. and Bendena, W. G. 1999. The regulation of juvenile hormone production

    in arthropods - functional and evolutionary perspectives. *Ann. N. Y. Acad. Sci.*

    897, 300-310.

Tomkins, J. L., Radwan, J., Kotiaho, J. S. and Tregenza, T. 2004. Genic capture and

    resolving the lek paradox. *TREE* 19, 323-328.

Tomkins, J. L., Kotiaho, J. S. and LeBas, N. R. 2005. Phenotypic plasticity in the

    developmental integration of morphological trade-offs and secondary sexual

    trait compensation. *Proc. R. Soc. Lond.* B 272, 543-551.

Truman, J. W. and Riddiford, L. M. 1999. The origins of insect metamorphosis.

    Nature 401, 447-452.

Truman, J. W. and Riddiford, L. M. 2002. Endocrine insights into the evolution of

    metamorphosis in insects. *Annu. Rev. Entomol.* 47, 467-500.

Trumbo, S. T. 1999. Using integrative biology to explore constraints on evolution.

    TREE 14, 5-6.

Trumbo, S. T., and Robinson, G. E. 2004. Nutrition, hormones and life history in

    burying beetles. *J. Insect Physiol.* 50, 383-391.

Tu, M. P. and Tatar, M. 2003. Juvenile diet restriction and the aging and reproduction

    of adult *Drosophila melanogaster. Ag. Cell* 2, 327-333.

Underwood, E. M., Caulton, J. H., Allis, C. D. and Mahowald, A. P. 1980.

    Developmental fate of pole cells in *Drosophila melanogaster Dev* . *Biol.* 77,

    303-314.

Van Noordwijk, A. J. and deJong, G. 1986. Acquisition and allocation of resources - their influence on variation in life-history tactics. *Am. Nat*. 128, 137-142.

Wagner, G. P. 1989. Multivariate mutation-selection balance with constrained pleiotropic effects. *Genetics* 122, 223-234.

Wheeler, D. E. 1991. The developmental basis of worker caste polymorphism in ants. *Am. Nat*. 138, 1218-1238.

Wheeler, D. E. and Nijhout, H. F. 2003. A perspective for understanding the modes of juvenile hormone action as a lipid signaling system. *Bioessays* 25, 994-1001.

Wilkinson, G. S. 1993. Artificial sexual selection alters allometry in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera, Diopsidae). *Genet. Res*. 62, 213-222.

Wilkinson, G. S., Fowler, K. and Partridge, L. 1990. Resistance of genetic correlation structure to directional selection in *Drosophila melanogaster Evolution* 44, 1990-2003.

Wilkinson, G. S. and Reillo, P. R. 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. *Proc. R. Soc. Lond*. B 255, 1-6.

Wilkinson, G. S. and Dodson, G. 1997. Function and evolution of antlers and eye stalks in flies. In *The evolution of mating systems in insects and arachnids* (ed. J. Choe and B. Crespi). Cambridge University Press. pp. 310-328.

Wilkinson, G. S., Kahler, H. and Baker, R. H. 1998. Evolution of female mating preferences in stalk-eyed flies. *Behav. Ecol*. 9, 525-533.

Wilkinson, G. S. and Taper, M. 1999. Evolution of genetic variation for condition-dependent traits in stalk-eyed flies. *Proc*. *R*. *Soc*. *Lond*. B 266, 1685-1690.

Wilkinson, G. S. and Sanchez, M. I. 2001. Sperm development, age and sex chromosome meiotic drive in the stalk-eyed fly, *Cyrtodiopsis whitei*. *Heredity* 87, 17-24.

Wilkinson, G. S., Swallow, J. G., Christensen, S. J. and Madden, K. 2003. Phylogeography of sex ratio and multiple mating in stalk-eyed flies from southeast Asia. *Genetica* 117, 37-46.

Wilkinson, G. S., Amitin, E. G. and Johns, P. M. 2005. Sex-linked correlated responses in female reproductive traits to selection on male eye span in stalk-eyed flies. *Integr*. *Comp*. *Biol*. 45, 500-510.

Wilson, T. G. 2004. The molecular site of action of juvenile hormone and juvenile hormone insecticides during metamorphosis: how these compounds kill insects. *J. Insect Physiol*. 50, 111-121.

Wilson, T. G., DeMoor, S., and Lei, J. 2003. Juvenile hormone involvement in *Drosophila melanogaster* male reproduction as suggested by the Methoprene-tolerant(27) mutant phenotype. *Insect Biochem*. *Mol*. *Biol*. 33, 1167-1175.

Wolfenbarger, L. L. and Wilkinson, G. S. 2001. Sex-linked expression of a sexually selected trait in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. *Evolution* 55, 103-110.

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

Yin, C. M., Qin, W. H. and Stoffolano, J. G. 1999. Regulation of mating behavior by nutrition and the corpus allatum in both male and female *Phormia regina* (Meigen). *J. Insect Physiol*. 45, 815-822.

Zahavi, A. 1975. Mate selection - selection for a handicap. *J. Theor. Biol*. 53, 205-214.

Zera, A. J., Potts, J. and Kobus, K. 1998a. The physiology of life-history trade-offs: Experimental analysis of a hormonally induced life-history trade-off in *Gryllus assimilis*. *Am. Nat*. 152, 7-23.

Zera, A. J., Sanger, T. and Cisper, G. L. 1998b. Direct and correlated responses to selection on JHE activity in adult and juvenile *Gryllus assimilis*: Implications for stage-specific evolution of insect endocrine traits. *Heredity* 80, 300-309.

Zera, A. J. and Harshman, L. G. 2001. The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst*. 32, 95-126.

Zhou, X. F., Coll, M. and Applebaum, S. W. 2000. Effect of temperature and photoperiod on juvenile hormone biosynthesis and sexual maturation in the cotton bollworm, *Helicoverpa armigera*: implications for life history traits. *Insect Biochem. Mol. Biol*. 30, 863-868.