

MALE AND FEMALE REPRODUCTIVE ROLES IN THE
CONTEXT OF LIFE HISTORY
AND NUTRITIONAL ECOLOGY

A dissertation submitted by

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In partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

in

Biology

TUFTS UNIVERSITY

August 2013

ADVISER: Dr. Sara M. Lewis

Abstract

Charles Darwin characterized sex roles as males pursuing and competing to gain females attention, and females acting coy and choosy in selecting their mates. Since then, the idea of “typical sex roles” has been a cornerstone for much of sexual selection theory. However, the last forty years have seen numerous studies showing that females in many species actively solicit multiple matings, and that males can be prudent and even picky when choosing a mate. Understanding the causes and consequences of these “non- typical” sex roles is an important part of contemporary biology. This dissertation uses a framework that incorporates testing adaptive and non-adaptive hypotheses to improve our knowledge of how non-typical male and female sexual traits and behaviors evolve.

The first part of this dissertation focuses on explaining sexual traits and behaviors in *Pieris rapae* butterflies, a species whose mating system includes nuptial gifts. My studies indicate that production of nuptial gifts by males, and acquisition of nuptial gifts by females are important drivers of non-typical sexual traits and behaviors. First, limitation of dietary nitrogen decreased female but not male allocation to primary reproduction. Low-nitrogen males sacrificed wing coloration, a trait associated with their mating success. *P. rapae* males might maximize fitness by protecting their investment in nuptial gifts and carefully choosing a mate. Supporting this idea I showed that males preferred to mate with more fecund females that had been reared in high-nitrogen diets. My studies also

indicated that male mate choice drives exaggeration of female coloration, a trait that honestly signals female fecundity in *P. rapae*.

Using an individual based simulation and knowledge based on lepidopteran biology, I demonstrated that male gifts can determine the magnitude of benefits that females obtain from allocating resources to ornaments. These simulations showed that females obtained maximum benefit by allocating almost half of their reproductive resources to ornaments and by acquiring multiple gifts.

In the last study presented in this dissertation, I used *Tribolium castaneum* flour beetles and applied sexually antagonistic selection on body size, which is an important trait for both male and female reproduction. My results showed that between-sex genetic correlations constrained the independent evolution of body size. These results support the idea that certain traits and behaviors, such as body size and ornamentation, can evolve as a correlated response to selection on the other sex.

Acknowledgements

First and foremost, I would like to thank Dr. Sara M. Lewis for her invaluable guidance and support during the completion of my dissertation work. Thanks Sara for helping me to grow not only as a scientist but also as a teacher and a mentor. Thanks for being so dedicated and patient with me, it was really a pleasure working with you. I would also like to thank my dissertation committee members, Dr. Philip Starks, Dr. Michael Reed, Dr. Colin Orians, Dr. Jan Pechenik and Dr. Frances Chew for providing thoughtful advice and assistance throughout the development of my doctoral research. I am especially grateful to Dr. Naomi Pierce, from Harvard University, who generously agreed to be a member of my committee. I am further grateful to Dr. Nathan Morehouse and Dr. Steven Levine, Jessica Chemali and Durwood Marshall for intellectual and technical guidance. I am also extremely grateful to the entire Biology Department for surrounding me with such a friendly and helpful atmosphere. This dissertation was made possible by funding through Tufts University Research Graduate Awards, Sigma Xi and U.S. Department of Agriculture. Lastly, special words of gratitude for my mother who always inspired me and encouraged me to dream and to pursue my dreams.

Table of Contents

Chapter 1. Introduction.....	1
Chapter 2. Linking nutrition and sexual selection across life stages in a model butterfly system.	7
<i>Published: Tigreros, N. 2013. Linking nutrition and sexual selection across life stages in a model butterfly system. Functional Ecology 27: 145-154</i>	
Chapter 3. Sex-specific responses to nutrient limitation and its effects on female mating success in a gift-giving butterfly	35
<i>Published: Tigreros, N. Sass M, & Lewis SM. 2013. Sex-specific responses to nutrient limitation and its effects of female mating success in a gift-giving butterfly. Evolutionary Ecology.</i>	
Chapter 4. Male mate choice favors more colorful females in the gift-giving cabbage butterfly.....	60
Chapter 5. Modelling sex-specific strategies for reproductive allocation: mate acquisition vs. nuptial gift/fecundity offspring investment.....	81
Chapter 6. Direct and correlated responses to artificial selection on sexual size dimorphism in the flour beetle, <i>Tribolium castaneum</i>	108
<i>Published: Tigreros N. & Lewis S. 2011. Direct and correlated responses to artificial selection on sexual size dimorphism in the flour beetle, Tribolium castaneum Journal of Evolutionary Biology 24: 835-842.</i>	
Chapter 7. Summary and conclusions.....	127

Index of Tables

Table 3.1. Results of generalized linear mixed model for the effect of larval dietary nitrogen on the mating success of female <i>P. rapae</i> butterflies.....	59
Table 4.1. Logistic regression models of the effect of male nutrition on probability of directing mating approaches towards a control vs. a pterin-extracted female.....	80
Table 5.1. Definitions for the parameters and variables in the models.....	107
Table 6.1. Linear regression slopes of cumulative responses to selection.....	122

Index of Figures

Figure 2.1. Effect of dietary nitrogen in development time and pupal size of <i>P. rapae</i> males.....	30
Figure 2.2. Summary of how dietary nitrogen during the larval stage of <i>P. rapae</i> males influenced adult phenotype and fitness	31
Figure 2.3. Effects of larval dietary nitrogen on wing color, latency to first mating and number of matings of <i>P. rapae</i> males.....	32
Figure 2.4. Effect of development time on total number of matings, spermatophore protein content and adult lifespan of <i>P. rapae</i> male..	33
Figure 2.5. Effect of pupal size on spermatophore size and number of sperm produced by <i>P. rapae</i> males.....	34
Figure 3.1. Effect of dietary nitrogen on developmental traits of male and female <i>P. rapae</i> butterflies.....	54
Figure 3.2. Effect of dietary nitrogen on allocation to adult structures by male and female <i>P. rapae</i> butterflies.....	55
Figure 3.3. Effects of dietary nitrogen on pterin-based wing coloration of <i>P. rapae</i> butterflies.....	56
Figure 3.4. Effect of dietary nitrogen on the mating success of <i>P. rapae</i> females.....	57
Figure 3. 5. Summary of how larval nitrogen limitation affects sex-specific resource allocation to adult structures when <i>P. rapae</i> butterflies.....	58
Figure 4.1. Spectra of control and pterin-extracted females.....	76
Figure 4.2. Relationship between potential fecundity and pterin-based wing coloration of <i>P. rapae</i> females.....	77
Figure 4.3. Percent of mating approaches of high-nutrition and low-nutrition <i>P. rapae</i> males toward females with either pterin-extracted or intact wings.	78

Figure 4.4. Total number of times that low-nutrition and high-nutrition <i>P. rapae</i> males approached a pair of females.....	79
Figure 5.1. Association between male and female allocation to different reproductive traits are male and female fitness.....	100
Figure 5.2. Description of the events that occur at each time step in a simulation model.....	101
Figure 5.3. Effects of female allocation to fecundity vs. mate acquisition on female fitness and mating success.....	102
Figure 5.4. Effects of male allocation to nuptial gifts vs. mate acquisition on male fitness and mating success.....	103
Figure 5.5. Effects of male allocation to nuptial gifts vs. mate acquisition on fitness obtained as a last and a past male.....	104
Figure 5.6. Effects of mating success on male and female fitness.....	105
Figure 5.7. Relationships between fecundity and mating success in males and females when males provide and do not provide nuptial gifts.....	106
Figure 6.1. Responses to artificial selection on pupal mass in the flour beetle <i>Tribolium castaneum</i>	124
Figure 6.2. Relationship between male and female pupal mass during seven generations of selection in <i>T. castaneum</i>	125
Figure 6.3. Correlated responses of developmental traits to selection on pupal mass in <i>T. castaneum</i>	126

Chapter 1: Introduction

In sexually reproducing species, males and females often pursue divergent reproductive strategies, investing differently in offspring production and care, as well as in secondary sexual traits and behaviors. Understanding the causes and consequences of these differing sex roles has been a main concern in the field of evolution and behavioral ecology.

While developing the principles of sexual selection, Charles Darwin (1859, 1871) was the first to thoroughly document “typical” sex roles: males generally pursue and compete to gain females’ attention, while females are coy and carefully choose their mates. Taking as examples plants and “lower animals”, Darwin proposed that these patterns of male and female sexual behaviors were due to differences in mobility between large ova and small sperm (p. 933). Inspired by Darwin’s ideas on sexual selection, Angus Bateman (1948) experimentally examined male and female reproductive patterns in *Drosophila melanogaster*. From these experiments Bateman concluded that only males benefited from promiscuity while female fitness was saturated after one or a few matings. Bateman believed that this difference was a result of the fundamental difference between the sexes in gamete size (anisogamy) and that the “greater dependence of males for their fertility on frequency of inseminations” was “an almost universal attribute of sexual reproduction” (p. 364). Darwin and Bateman’s ideas were further extended by Trivers (1972, 1985). Trivers proposed that sex roles were a result of differences in male and female parental investment. He

considered parental investment as a composite of investment in gametes as well as post-zygotic investment (parental care).

These ideas have been a cornerstone for much of sexual selection theory. Great number of studies in sexual selection has examined how female choice and male competition select for exaggerated secondary sexual traits in males. However, more recent work on male and female sexual behaviors and reproductive costs does not always accord with the idea of typical sex roles. The literature provides evidence for many exceptions, including females that actively solicit matings with multiple males (e.g. Gowaty et al. 2003), males that do not always mate indiscriminately (reviewed in Bonduriansky 2001) and females that bear ornaments (e.g. LeBas et al. 2003). As a result, the longstanding ideas of typical sex roles have been challenged, and it has been acknowledged that male and female roles are more flexible than what was originally proposed (Gowaty 2003).

Several new ideas have been proposed to explain the causes and consequences of “non- traditional” sex roles (Bonduriansky 2009). These are now an important part of contemporary biology, and their incorporation into theoretical and empirical studies will further our knowledge about the evolution of male and female reproductive roles. Explanations for non-traditional sex roles include both adaptive and non-adaptive hypotheses and are discussed below.

One adaptive explanation for why females in many species mate multiple times and often solicit matings (e.g. Hardy 1997) proposes the existence of direct

and indirect mating benefits. Indirect benefits include high genetic quality and diversity of a female's offspring (Yasui 1998), while direct benefits include renewal of depleted sperm supplies, increased access to resources, and increased lifetime offspring production (Arnqvist 2000; Birkhead, 2000). Males in many taxa provide females with a nuptial gift during mating (Lewis et al. 2011), and such gifts can increase a female's lifetime fecundity (Boggs 1995; Lewis and South 2012). Because of the direct benefit that these nuptial gifts provide, females should increase nuptial gift acquisition by mating multiple times. As a consequence, female traits and behaviors that improve mating success are expected to evolve (e.g. female mate solicitation, female ornaments and weapons).

In many species, males have been shown to be prudent and even picky when choosing a mate (Bonduriansky 2001). Males have been described as being so choosy that they simply refuse to mate when solicited by certain females (e.g. Pinxten and Eens 1997; Gowaty et al. 2003). It is now well established that because male reproductive capacity is often limited, males might be selected for prudent allocation of their ejaculates (Dewsbury 1982). For example, male production of nuptial gifts is costly and in some species can represent between 1.5 and 24% of male body mass (Svärd and Wiklund 1989). In such systems, male mate choice is expected to evolve (Bonduriansky 2001).

On the other hand, non-adaptive explanations have been also proposed to account for features such as female ornaments. In species with traditional sex roles, sexual selection is expected to act more strongly in males than in females,

and as a consequence, the expression of exaggerated traits and behaviors tend to be greater in males than in females (Andersson 1994). When females exhibit exaggerated traits, their evolutionary significance has proven to be enigmatic. One hypothesis views the existence of female exaggerated traits and behaviors as a non-adaptive, correlated response to selection on male traits (Lande 1980; Lande and Arnold 1983). Because males and females share most of their genes, genetic correlations between the sexes can be high (Lande 1980, Lande and Arnold 1983) and constrain each sex from reaching independent trait optima (Lande 1980; Roff 1997). Understanding whether homologous traits in the two sexes are free to evolve independently of each other is necessary to explain the differences between male and female reproductive roles. It is important to determine the extent to which between-sex genetic correlations might constrain the independent evolution of traits in each sex.

This dissertation uses a framework that incorporates testing adaptive and non-adaptive hypotheses to improve our understanding of how non-traditional male and female sexual traits and behaviors can evolve. Using life history and nutritional ecology theory, I chose to work with insects with a complex life cycle (beetles and butterflies). These creatures are characterized by discrete larval and adult stages that often live in distinct habitats with different nutritional conditions. The larval stage is dedicated to acquiring most of the resources necessary to shape the adult and to reproduce. Differences in resource availability during early life history stages are expected to have major consequences for adult reproduction.

Because of their different reproductive roles, males and females are expected to have different nutritional requirements, and different responses to early food limitation. Thus, studying sex-specific responses to nutritional constraints should help us better understand differences in male and female reproductive roles.

This thesis is organized into seven chapters. Chapters 2 through 5 examine different aspects of male and female nutrition and reproduction in a gift-giving butterfly, *P. rapae*. Like all insects with complete metamorphosis, *P. rapae* butterflies acquire most of the resources they need to grow, survive, and reproduce during their larval stage. *Pieris* larvae eat a variety of crucifer plant species which significantly vary in their nitrogen content (1.9 – 4.9 %) (Morehouse 2009; Slansky & Feeny 1977). Fitness of both males and females requires allocation of nitrogen to various traits. Males transfer a protein-rich package called spermatophore (Bissoondath & Wiklund 1995) which increases female life-time fecundity (Boggs & Gilbert, 1979), and male reproductive success (Cook & Wedell, 1996; Wedell & Cook, 1998). Male and female color is based on pterins, a nitrogen-rich pigment (Kayser 1985). Allocation to wing pterins might impact both male and female fitness, since females prefer to mate with males with more colorful wings (Morehouse & Rutowski 2010) and males discriminate between the sexes based on wing coloration (Arikawa et al. 2005). Although other fitness traits, like male pheromone production (Yildizhan et al. 2009) might also be affected by dietary constraints, I am focusing this work to the effects on wing coloration, female fecundity and male nuptial gifts.

Chapter 2 shows how larval nutrition impacts key aspects of male reproductive success. These experiments measured the effects of larval nitrogen limitation on a comprehensive set of traits associated with both male mating success and nuptial gift quality. Chapter 3 studies sex-specific responses to food limitation, focusing on how this impacts female mating success and gift acquisition. Chapter 4 tests for the evolution of male mate choice and female honest ornamentation. This study uses experimental manipulation of female ornaments (wing pterin-based coloration), as well as a correlational assessment of the relationship between wing coloration and egg load in field-collected females. Chapter 5 uses individual based simulations and knowledge based on the lepidopteran biology to understand how the optimal allocation strategy to different reproductive traits in one sex depends on the allocation pattern shown by the other sex. Chapter 6 uses *Tribolium castaneum* flour beetles to test whether body size (a key trait for both male and female reproductive success) is free to evolve independently in males and females. To do this I performed an artificial selection experiment where I subjected males and females to sexually antagonistic selection on body size (sexes selected in opposite directions). Finally, chapter 7 synthesizes and discusses the results of studies described in the preceding chapters.

Chapter 2: Linking nutrition and sexual selection across life stages in a model butterfly system

Summary

1. Early nutrition plays an important role in determining adult fitness. Theory proposes that in organisms with complex life-cycles, the fitness effects of larval nutritional constraints are mainly captured by two developmental traits: time and size at metamorphosis. However, recent evidence suggests that latent effects, which are independent of these developmental traits, must be included in order to fully understand how larval nutrition impacts fitness.

2. In this study I used the cabbage butterfly (*Pieris rapae*) as a model system to investigate how larval nutrition influences male fitness through development time, adult size, and latent effects. Specifically I examined how variation in dietary nitrogen impacts male fitness by measuring a comprehensive suite of pre- and post-copulatory traits.

3. Results indicate a complex role for larval nutrition in determining adult fitness. Larval nutritional constraints influence adult fitness through three different pathways: development time, adult size and latent effects. Longer development times were associated with shorter adult lifespan and reduced male mating success. Body size was positively correlated with traits related to post-copulatory fitness, such as spermatophore size and protein content. Larval nitrogen availability, independent of the developmental traits, also affected traits

associated with male mating success such as wing coloration and latency to first mating.

4. These results provide new insights into how larval nitrogen availability alters adult fitness by revealing novel links between larval dietary nitrogen and various adult fitness components.

Introduction

Animals and plants grow and reproduce surrounded by nutritional variation, where food is often scarce or key nutrients are lacking. Because the juvenile nutritional environment has major effects on the adult phenotype, linking nutrition and fitness is an increasingly important aspect of ecology, evolution, and life history theories (e.g. Boggs 2009; Raubenheimer, Simpson & Mayntz 2009; Morehouse et al. 2010).

Developing under nutritional constraints can be particularly challenging for animals with a complex life-cycle, such as amphibians, holometabolous insects and many marine invertebrates. Such creatures are characterized by discrete larval and adult stages that often live in distinct habitats with different nutritional conditions. Thus, individuals have limited time to acquire the resources that are necessary to form the adult and to reproduce. Traditionally, life history models propose that the effects of larval nutrition on adult fitness are mainly captured by two developmental traits: size and timing of transition out of the larval stage (e.g. Rowe & Ludwig 1991; Moran 1994; Awmack & Leather 2002; Roff 2002).

Typically, nutrient limitation produces longer development time and/or smaller body size at metamorphosis, and these changes in developmental traits are correlated with reduced adult fitness. However, recent studies challenge these models by showing that larval nutrition also impacts adult fitness independently of these developmental traits (e.g. De Block & Stoks 2005; Stoks, De Block & McPeck 2006; De Block & Stoks 2008). This occurs through “carry over” or latent effects, in which traits originate from the larval nutritional experience yet are only expressed in adulthood (Pechenik, Wendt & Jarrett 1998). Examples of latent effects due to restricted larval diets include decreased adult immune function (Fellous & Lazzaro 2010), adult shortage of energy storage molecules (Stoks, De Block & McPeck 2006) and increased oxidative stress (De Block & Stoks 2008). Thus, by taking into account these additional latent effects we can achieve a more complete understanding of how early nutrition might affect various components of adult fitness.

A second important issue when examining how juvenile nutrition alters adult fitness concerns the methods used to evaluate fitness. Measuring fitness is a challenging enterprise, especially because male fitness often depends not only on mating success, but also on different pre- and post-copulatory fitness components (Hughes 1998; Fedina & Lewis 2008). When resources are limited, fitness variation among individuals arises through different resource allocation patterns among various fitness components (Stearns 1992). However, the vast majority of studies looking at the fitness effects of larval nutrition have only focused on traits

related to either pre-copulatory or post-copulatory fitness; remarkably few studies have considered both (but see Lewis, Sasaki & Miyatake 2011; Lewis et al. 2012). A more complete understanding of how nutrition affects fitness requires taking into account both pre- and post-copulatory fitness. An additional challenge is that fitness is ideally measured under natural conditions where keeping track of individuals is often a difficult task. Therefore, it is by considering both pre- and post copulatory fitness components and by measuring these components in settings reflecting natural conditions that we obtain more precise estimates of fitness.

Herbivorous insects are an appropriate study system for examining the linkages between nutrition and fitness. Nitrogen is a key nutrient for all animal species because it is required to build proteins, nucleic acids, and many essential body structures (Mattson 1980; Bernays & Chapman 1994). However, because plant tissue contains only a small fraction of the nitrogen contained in animal tissue, nitrogen becomes a limiting element for most herbivores (Mattson 1980, Scriber & Slansky 1981; White 1984; Slansky & Rodriguez 1987; Bernays 1998; Awmack & Leather 2002). This results in a fundamental nutritional mismatch between herbivores and their food plants; caught in what has been called the herbivore's dilemma (Pierce & Berry 2011), these creatures must reconcile their nitrogen-rich lifestyle with their nitrogen-poor diet. The cabbage butterfly, *Pieris rapae*, is a model organism for studying the role of nitrogen limitation because of its particularly high demands for nitrogen, with adult bodies consisting of ~13% nitrogen at eclosion (Morehouse & Rutowski 2010a). This butterfly is also useful

for testing how larval nutrition affects adult fitness because several male traits related to pre- and post-copulatory fitness components have previously been identified (e.g. Suzuki et al. 1977; Bissoondath & Wiklund 1996; Wedell & Cook 1999; Morehouse & Rutowski 2010b). Finally, Morehouse & Rutowski (2010a) found that larval nitrogen availability strongly affected larval growth and development. These authors also suggested that dietary nitrogen influences key male adult traits including male ornaments such as wing coloration and nutritive nuptial gifts that are passed to the females during copulation (Morehouse 2009; Morehouse & Rutowski 2010a). In this species, females prefer to mate with more colorful males (Morehouse & Rutowski 2010b) and wing coloration is based on pterins, a group of nitrogen-rich pigments (Kayser 1985). *P. rapae* males' nuptial gift consists of a protein-rich spermatophore (Bissoondath & Wiklund 1995) that increases male reproductive success by increasing female fecundity (Watanabe & Ando 1993), remating latency (Sugawara 1979; Kandori & Ohsaki 1996) and male paternity share (Wedell & Cook 1998).

In this study, I adopt a holistic approach to address the longstanding question of how adult fitness of an herbivorous insect is affected by the larval nutritional environment. I manipulated larval dietary nitrogen availability and measured its effects on adult fitness through three different pathways: development time, adult size and latent effects. In addition, I measured a suite of adult male fitness components in a setting that approximates natural conditions for the cabbage butterfly. Using this approach, I expected to gain a more comprehensive picture of

how dietary nitrogen influenced male adult fitness in an organism with a complex life cycle.

Materials and methods

DIETARY NITROGEN MANIPULATION

Nitrogen is a key limiting nutrient in herbivores, such as butterflies, and variation in the availability of this nutrient is expected to be tightly linked with variation in organismal fitness. Host plants of the cabbage butterfly naturally vary in nitrogen content ranging from 1.9% to 4.8% nitrogen (Slansky and Feeny 1977; Morehouse & Rutowski 2010a). In this experiment I manipulated the level of dietary nitrogen using a semi-synthetic diet following methods used in Morehouse & Rutowski (2010a). Briefly, I manipulated the levels of nitrogen by replacing vitamin-free casein, the primary source of protein in this diet, with equivalent amounts of cellulose, which is a non-nutritive and nontoxic filler. This manipulation altered nitrogen concentration while leaving all the other nutrients constant. Seven semi-synthetic diets were prepared with different nitrogen percentages that represented the natural nitrogen range, including one “standard” diet (3.5%) and six “treatment” diets with percentage nitrogen by dry mass of: 2.0, 2.4, 2.8, 3.2, 3.7, and 4.1%.

All larvae were reared from hatching to 10 d (late 2nd or early 3rd instar) on the standard diet (3.5% nitrogen), at which time experimental males were switched to one of the six treatment diets. Females used in this experiment were reared on diets with similar nitrogen levels (3.2, 3.5 or 3.7%).

EXPERIMENTAL INDIVIDUALS

Butterflies in this experiment were the F1 offspring of 9 singly-mated females reared from eggs obtained commercially (Carolina Biological Supply Company, Burlington, NC). For the goals of this experiment I needed to rear larvae from different nitrogen diets and yet get all adults to emerge at approximately the same time. Because decreasing nitrogen increases larval development time (Morehouse & Rutowski 2010a; Tigreros unpublished) I synchronized adult emergence by starting individuals assigned to the lowest nitrogen diets first. The offspring from at least 3 different females were assigned to each diet treatment and each female contributed eggs to at least 2 diet treatments. Females' first batch of eggs were assigned to several different (low and high nitrogen) diets.

Individuals were kept from egg to adult in individual petri dishes (Fisher Scientific, Houston, Texas) stored in an incubator with an 18L: 6D photoperiod and 25°C: 20°C at 60% relative humidity. Larvae were provided with cubes of diet which were replaced every four days. All adults were fed with a 25% honey solution and stored at ~8 °C until the mating trial.

MEASURED VARIABLES

Developmental traits: I measured development time as the number of days from egg hatching to pupation. Pupal size was quantified as wet mass measured during the 3rd day after pupation.

Adult phenotypic traits: I measured aspects of the adult phenotype known to correlate with different fitness components. These included wing size and coloration, both important in courtship and mate acquisition, and testis size, important in sperm production. Because these traits may change over an individual's lifetime, I measured these in a subset of adults that were frozen immediately after emergence. To measure wing size I photographed the right fore- and hind-wings and measured their area using ImageJ 1.45 software. Pterin-based wing coloration was measured according to Morehouse & Rutowski (2010b) protocols. Briefly, I measured the spectral reflectance of a 2 mm area on the dorsal left forewing using a JAZ Ocean Optics spectrophotometer. From the measured spectra, I calculated four parameters that have been shown to be relevant to both pterin coloration and female vision (Morehouse & Rutowski 2010b): $R_{300-375}$, $R_{550-650}$, λ_{R50} , β_{R50} . These four parameters were reduced using a principal component analysis into one single variable, PC1: this first principal component explained 99.9% of the variation in pterin-based wing coloration.

To measure testis size, both testes were dissected, dried and weighed to the nearest 0.1 μ g using a Mettler Toledo MT5 microbalance (Columbus, Ohio).

Male mating success: One important male fitness component is the ability to attract and successfully mate with females. Male mating success should ideally be measured in natural or seminatural conditions where male-male competition and female rejection and acceptance behaviors can be freely displayed. I measured male mating success in such conditions by releasing adult butterflies into an

outdoor insectary (20 m long, 3 m height, 3 m wide) large enough to keep a natural adult density (~ 1.7 individuals/m³). Within this enclosure, adults could fly, court and reject potential mates. A similar approach used in other studies to measure adult fitness in both butterflies (Bergman, Gotthard & Wiklund 2011) and damselflies (De Block & Stoks 2005). The insectary was located on the Tufts University campus where flying adults of *P. rapae* are normally seen. During the trial, temperatures ranged from 27°C to 35°C, and butterflies were provided with cabbage (host plant), damp sponges and flower bouquets.

A total of 37 females and 68 males (6 to 17 males per diet treatment) were simultaneously released in the outdoor insectary on July 2010. Prior to their release, males were individually marked by placing four colored dots (using Sharpie Extra Fine markers) near the base of the forewing on the costal margin area. These marks were very small (~ 0.8 mm diameter) and microscopic examination showed they did not damage wing scales. Although the number of males decreased over the course of the experiment, the number of females in the insectary was also changed to maintain a 2:1 sex ratio. This sex ratio matches that typically found in the beginning of the mating season, when males are most competitive and when most virgin females are found (Wiklund, Wickman & Nylin 1992).

The insectary was continuously patrolled from 06:30h to 17:30h, these butterflies' active mating period, for a total of 5 days. During this period of continuous observation, I measured latency to first mating and mating success for each male by recording the date and time when each male mated. Mating pairs

were collected in plastic cups, male ID was determined and the male was released back into the insectary after the end of copulation. Mated females were sacrificed for later dissection and measurement of the male's spermatophore; each female removed was replaced with a new virgin female.

Spermatophore quality: Like other Lepidoptera, male *P. rapae* transfer a spermatophore during each mating (Wedell 2005). This spermatophore is a package that contains sperm as well as significant amounts of protein and other nutrients (Marshall 1982). Spermatophore quality, in terms of size, protein content, and sperm number, is an important determinant of a male's post-copulatory fitness in pierid butterflies (e.g. Boggs & Watt 1981; Bissoondath & Wiklund 1995; Wedell & Cook 1998). In *P. rapae*, large spermatophores increase female egg laying (Watanabe & Ando 1993), female remating latency (Sugawara 1979; Kandori & Ohsaki 1996), and increase male paternity share in competitive mating situations (Wedell & Cook 1998). In this study I measured wet mass, protein percent and sperm number contained within a male's first and second spermatophores. To do this, mated females were dissected within 20 minutes of finishing copulation, before sperm began to migrate out of the spermatophore (Cook & Wedell, 1996); female bursas containing the spermatophore were stored individually in 80% ethanol for later analysis. Later, male spermatophores were carefully dissected out and their size determined as wet mass measured to 0.1 ug accuracy (Mettler Toledo MT5 microbalance, Columbus, Ohio). Within the spermatophore the sperm ampulla consists of a small compartment containing two

types of sperm: eupyrene (nucleated) and apyrene (anucleate sperm) sperm. In this study I counted eupyrene sperm by first carefully separating the sperm ampulla from the rest of the spermatophore, opening the ampulla and counting eupyrene sperm bundles at 40X magnification; the number of bundles was multiplied by 256, the total number of sperm contained in a bundle, to obtain the total number of eupyrene sperm (Cook & Wedell 1996). The remaining part of the spermatophore (without sperm) was used for protein analysis using the Bradford method (Bradford 1967) with a BioRad protein assay (Bio-Rad Laboratories, Hercules, California) following methods in Bissoondath & Wiklund (1995).

Adult lifespan: *P. rapae* adults live for about 2-3 weeks. Adult lifespan was measured for a period of 12 days, starting on the day when males were first released into the insectary. Prior to this time, emerging adults were kept at ~8°C to slow metabolism. Males that remained alive after the observation period in the insectary were kept in an incubator (18L: 6D photoperiod and 25°C: 20°C at 60% relative humidity) to record survival over 7 additional days. After that time, remaining males were sacrificed and their lifespans included as truncated data.

DATA ANALYSIS

To examine the effects of dietary nitrogen on development time and pupal size I used two separate linear regressions for development time and pupal size. Then, I used a series of models to estimate the independent effects of dietary nitrogen, developmental time and pupal size on each adult trait.

The analysis of each model consisted of first using a generalized additive model (Proc GAM, SAS ver. 9.1) to identify the appropriate form of dependence by applying nonparametric regression and smoothing techniques. Second, each model was analyzed using a generalized mixed linear model (GLIMMIX macro in SAS ver. 9.1) using the particular form (linear, quadratic, etc.) suggested by the GAM procedure. Results are presented from the GLIMMIX analysis which controls for possible non-independence of males within families by including individual males as the random factor blocked by family.

The distribution specified in the GLIMMIX models for wing size, wing color, testis size, lifespan and latency to first mating was normal. A Poisson distribution was used in GLIMMIX for mating success. Because lifespan included truncated data, I used the Cox proportional hazards model (PHREG procedure, SAS ver. 9.1) which allows inclusion of censored data.

Results

EFFECT OF DIETARY NITROGEN ON DEVELOPMENTAL TRAITS

Larval dietary nitrogen level affected both development time and pupal size of *P. rapae* males. Increased nitrogen concentration resulted in significantly shorter development time (Fig. 2.1a; linear regression, $F = 23.28$ $P < 0.0001$, $N = 220$) and larger pupal size (Fig. 2.1b; $F = 19.5$ $P < 0.0001$, $N = 220$). The effect of dietary nitrogen was stronger for development time ($R^2 = 0.41$) than for pupal mass ($R^2 = 0.17$).

ADULT MORPHOLOGICAL TRAITS

Dietary nitrogen concentration, independent of pupal size and development time, did not affect either male fore-wing area (GLIMMIX, $F_{1,44} = 3.01$, $P = 0.09$) or hind-wing area ($F_{1,54} = 0.91$, $P = 0.34$). Also, testes size was not affected by larval nitrogen concentrations ($F_{1,42} = 2.31$, $P = 0.14$). In contrast, adult wing coloration was significantly altered by nitrogen concentration (Fig. 2.2, Fig. 3a; $F_{1,61} = 30.10$, $P < 0.0001$) independent of the developmental traits. Adult morphological traits covaried with variation in pupal mass (Fig. 2.2); males with greater pupal mass had larger fore-wing areas ($F_{1,44} = 178.17$, $P < 0.0001$), hind-wing areas ($F_{1,52} = 181.93$, $P < 0.0001$), and larger testes sizes ($F_{1,42} = 24.76$, $P < 0.0001$). Male pupal mass was also positively related to wing coloration ($F_{1,59} = 14.79$, $P = 0.0003$).

On the other hand, variation in development time did not influence adult phenotypic traits (fore-wing area $F_{1,44} = 0.01$, $P = 0.91$; hind-wing area: $F_{1,54} = 2.71$, $P = 0.10$; testis size: $F_{1,42} = 0.62$, $P = 0.44$; wing color $F_{1,59} = 1.18$, $P = 0.28$).

ADULT FITNESS TRAITS

Male mating success

During the 5 days of continuous observation, 50 out of 68 males mated at least once. For these males, the time between when they were released into the mating arena and the first mating (latency to first mating) ranged from 15 min to 1.5 d, and this variation was significantly related to dietary nitrogen concentration (Fig.

2.2, $F_{1,38} = 5.41$, $P = 0.02$). Surprisingly, males reared on lower nitrogen diets achieved their first mating sooner than those reared on higher nitrogen diets (Fig. 2.3b). Yet male latency to first mating was not affected by either of the developmental traits (pupal mass: $F_{1,38} = 1.08$, $P = 0.31$; development time: $F_{1,38} = 0.01$, $P = 0.94$).

Overall, *P. rapae* males averaged 1.8 ± 0.21 matings (median = 1.5) during the 5 days of observation, with a range from 0 to 7. Interestingly, there appeared to be an almost significant quadratic effect of dietary nitrogen concentration on total number of matings, with males reared on the low and high nitrogen diets both averaging more matings per male (Figs 2.2 and 3c; $F_{1,30.13} = 4.05$, $P = 0.0502$). Total number of matings was also influenced by variation in development time (Fig. 2.2): males developing more quickly generally mated more often than those with longer development times (Fig. 2.4a; $F_{1,42.35} = 11.25$, $P = 0.002$). However, there was no effect of pupal mass on total mating number ($F_{1,56.63} = 0.81$, $P = 0.37$).

Spermatophore quality

During their first mating, *P. rapae* males transferred spermatophores that weighed 4.39 ± 0.13 mg (N= 48, range 1.73- 5.85 mg wet mass). During second matings, spermatophore weight declined significantly to 1.73 ± 0.17 mg (n = 30, range 0.4- 3.9 mg) (Paired t test, $t = 16.15$, $P < 0.0005$, N = 27). Spermatophore protein content expressed as percent of total wet mass also decreased from $1.65 \pm 0.16\%$ in first spermatophores to $0.3 \pm 0.07\%$ in second spermatophores (Paired t test, $t = 6.96$, $P < 0.0005$, N = 27). However, no significant difference was detected in

number of eupyrene sperm transferred in first (11620 ± 1071) compared to second spermatophores (12604 ± 1058) (Paired t test, $t = -0.3$, $P = 0.7$, $N = 12$).

Male spermatophore mass was not affected by either dietary nitrogen (first spermatophore: $F_{1,36} = 0.77$, $P = 0.39$; second spermatophore: $F_{1,21} = 1.19$, $P = 0.29$) or development time (first spermatophore: $F_{1,36} = 0.61$, $P = 0.44$; second spermatophore: $F_{1,21} = 2.83$, $P = 0.11$). However, males with large pupal size produced significantly larger spermatophores (Figs 2.2 and 2.5a; first spermatophore: $F_{1,36} = 39.96$, $P < 0.0001$; second spermatophore: $F_{1,21} = 9.41$, $P = 0.006$).

The protein concentration of male spermatophores was not altered by either dietary nitrogen (first spermatophore: $F_{1,36} = 0.81$, $P = 0.37$; second spermatophore: $F_{1,20} = 1.09$, $P = 0.31$) or by pupal mass (first spermatophore: $F_{1,36} = 3.24$, $P = 0.08$; second spermatophore: $F_{1,20} = 0.78$, $P = 0.39$). Surprisingly, males with longer development times produced first spermatophores with higher protein content than did males with shorter development times (Figs 2.2 and 2.4b; $F_{1,36} = 5.17$, $P = 0.03$). However, development time was not significantly related to protein concentration of males' second spermatophores ($F_{1,20} = 0.85$, $P = 0.37$).

In addition, there was a non-significant trend for males reared in low nitrogen diets to produce second spermatophores containing fewer sperm (Figs 2.2 and 2.5b; $F_{1,13} = 4.39$, $P = 0.056$). Nevertheless, even with a larger sample size, no effect of nitrogen was detected for sperm content of first spermatophores ($F_{1,23} = 1.46$, $P = 0.24$). Neither of the developmental traits was correlated with number of sperm

contained within males' first or second spermatophores. Development time did not correlate with sperm number within male's first ($F_{1,13} = 0.35$, $P = 0.5$) or second spermatophores ($F_{1,23} = 0.01$, $P = 0.97$), nor was pupal size correlated with sperm numbers (first spermatophore: $F_{1,13} = 2.17$, $P = 0.16$; second spermatophore: $F_{1,23} = 2.11$, $P = 0.16$).

Adult lifespan

Interestingly, development time influenced adult male lifespan (Fig. 2.4c; PHREG: $\chi^2 = 8.68$, $df = 1$, $P = 0.003$): males with longer larval development times had significantly shorter adult lifespans. However, adult lifespan was not affected by either larval dietary nitrogen ($\chi^2 = 2.31$, $df=1$, $P = 0.13$) or pupal size ($\chi^2 = 0.74$, $df = 1$, $P = 0.39$).

Discussion

Nutritional constraints early in life are predicted to have important consequences for adult fitness. This study offers one of the most complete views of how larval nutrition affects fitness by including a comprehensive suite of pre-and post-copulatory traits and by examining the effects of larval diet acting not only through key developmental traits, but also independently of them. The main findings are discussed below.

EFFECT OF NITROGEN ON DEVELOPMENTAL TRAITS

As expected based on previous studies (Wolfson 1982, Chen et al. 2004, Hwang et al. 2008, Morehouse 2010a), decreasing dietary nitrogen significantly lengthened development time. Nitrogen also affected pupal mass but to a lesser degree, which may explain why pupal mass has been often found to be unaffected by nitrogen (Chen et al. 2004; Morehouse 2010a). Thus, these results suggest that when larval nitrogen availability is reduced, *P. rapae* males tend to increase their development time and maintain their body size.

INDEPENDENT EFFECTS OF NITROGEN ON ADULT FITNESS

Larval dietary nitrogen, independent of development time and pupal mass, had significant effects on adult phenotypic traits and fitness of *P. rapae* males. First, dietary nitrogen was positively related to a male's sexual signal, wing coloration, supporting the hypothesis that dietary nitrogen constrains male allocation to nitrogen-rich pterin pigments (Morehouse & Rutowski 2010b). Morehouse & Rutowski (2009) used a similar diet manipulation to examine the effects of genotype and dietary nitrogen in *P. rapae*, but did not detect a main effect of nitrogen on male wing coloration. Instead, they showed that wing coloration was affected by the interaction between diet and genotype. Thus, these two studies illustrate the importance of dietary nitrogen availability in enhancing male wing coloration in some genotypes of *P. rapae* butterflies.

Results of this study showing that dietary nitrogen alters male wing coloration suggest that this male trait may provide information on the quality of a male's

nutritional environment and therefore signal male phenotypic condition. Previous studies have proposed that male wing coloration in the Pieridae may be an honest signal of direct benefits such as spermatophore quality (Kemp & Rutowski 2007; Morehouse 2009). If this is true, both the signal and the benefit would be predicted to vary as a function of diet. Although I found that wing color reflected larval nutritional status, most measured aspects of spermatophore quality were unaltered by larval diet. In this study wing coloration and spermatophore quality were assessed in different individuals (sib-related), thus I cannot directly assess the relationship between these two traits. Studies directly assessing the relationship between male wing coloration and spermatophore quality are necessary to gain more insight into the evolution of wing coloration as an honest signal of direct benefits.

These results also revealed that larval dietary nitrogen influenced male mating success in two distinct ways. First, males reared on low nitrogen diet tended to mate sooner than those in the nitrogen rich diets. For species such as *P. rapae* with a short adult lifespan (Moreau, Benrey & Thiery 2006) and a high risk of sperm competition (Simmons et al. 1994), how soon a male begins mating has important fitness consequences. A short latency to first mating might increase both a male's chance of mating at all before death and his chance of mating with a virgin female, thus avoiding sperm competition with a female's previous mates. Low nitrogen males might be able to achieve a short latency to first mating by being less choosy or having a faster sperm production rate than males reared in high nitrogen diets.

Thus, by decreasing their mating latency, low quality males might compensate for disadvantages in other fitness-related traits such as being less attractive or producing few sperm. This study also suggests that dietary nitrogen increases mating success of both low and high nitrogen males, which highlights the importance of examining diet effects using a diet gradient that allows detection of nonlinear effects. High mating success is expected for males in high nitrogen diets, which are likely to be the most attractive males (e.g. in terms of wing coloration). For example, Morehouse & Rutowski (2010b) showed that *P. rapae* females prefer to mate with more colorful males. Conversely, the high mating success of males in low nitrogen diets is a surprising result, indicating that males reared under nitrogen constraints somehow buffer the effects of limited nitrogen. A possible explanation for this result is that these low nitrogen males achieve high mating success by being less choosy. In *P. rapae*, not only females but also males exhibit mate choice (Obara, Koshitaka & Arikawa 2008; Obara et al. 2008; Tigreros unpublished). In some species the degree of choosiness is variable, with low quality individuals being less choosy than high quality individuals (e.g. Holveck, Geberzahn & Riebel 2011). This idea that males reared on low nitrogen diets are less choosy is also supported by the finding that these males also initiated mating sooner than did males reared on high nitrogen diets. In short, while high nitrogen males may increase their mating success by being more attractive to females, low quality males may increase their mating success by being less choosy.

EFFECTS OF DEVELOPMENTAL TRAITS ON ADULT FITNESS

Development time, which was strongly affected by variation in dietary nitrogen, influenced several components of male fitness: total number of matings, protein content of a male's first spermatophore, and adult lifespan. Conventionally, short development times have been thought to provide a fitness advantage by reducing the risk of death before reproduction (Stearns 1992; Sibly & Calow 1986). Nevertheless, in this study I detected additional fitness effects of development time that have not been traditionally considered. First, I found that shorter larval development times were associated with longer male adult lifespan and higher mating success. Although a connection between time spent as larvae and adult lifespan has been rarely considered, this result is congruent with the genetic tradeoff between larval development time and adult lifespan shown in another lepidopteran, the butterfly *Bicyclus anynana* (Pijpe et al. 2006). The relationship between larval development time and mating success is probably a consequence of males living longer as adults and therefore having more chances to mate. On the other hand, males that took a long time to develop allocated greater amounts of protein into their first spermatophore than those males with shorter development. In the cabbage butterfly, as in many other species, males can increase their own reproductive fitness by transferring a spermatophore that enhances their mate's reproductive output (Watanabe & Ando 1993). Since protein is an essential component of oogenesis (Chapman 1998), a high investment in spermatophore protein might increase reproductive fitness. Taken together, these results suggest

two alternative strategies linked to larval development time through which males could increase their reproductive success. On one hand, males with rapid larval development will live longer as adults and thus will acquire more matings. On the other hand, males with slow larval development, although they have short adult lifespans, will produce high quality spermatophores (high protein concentrations) in their first mating.

Male body size is often considered to impact pre-copulatory success, with larger males benefitting through female choice or male-male competition. Although several aspects of male phenotype which could potentially influence mate acquisition ability (e.g. wing area and wing color) were found in this study to be influenced by pupal size, pupal size did not directly alter male mating success (latency to first mating or number of matings). Nevertheless, variation in pupal size did influence traits that are linked with a male's post-copulatory success. As found in other studies (Bissoondath & Wiklund 1996; Wedell & Cook 1998), males with larger pupal size produced larger spermatophores that contained more protein. These two components are important in the male's post-copulatory performance in several ways. First, larger spermatophores increase female refractory periods before remating (Sugawara 1979; Kandori & Ohsaki 1996). Second, large and protein-rich spermatophores might increase female reproductive output (Watanabe & Ando 1993) and third, males with large spermatophore are able to sire more offspring even when their sperm is competing with other males' sperm (Wedell & Cook 1998). This study supports the contention that variation in

body size due to nutritional constraints might play an important role not only in the outcome of pre-copulatory but also post-copulatory processes (e.g. McGraw et al. 2007).

In summary, while a large body of literature shows that larval nutrition is an important determinant of adult fitness, here I show that the role of nutrition is considerably more complex than what has been commonly thought. First, as pointed out by De Block & Stoks (2005) larval constraints are not entirely captured by the developmental traits of development time and size at pupation. Larval nutrition influenced adult fitness through three different pathways: by altering time to metamorphosis, by altering pupal size (size at transition) and through nutritional latent effects carried over into the adult independently of time and size at metamorphosis. Second, the impact of larval diet on adult fitness (through any of the mentioned pathways) was neither entirely positive nor negative. Instead, some fitness components were positively and others were negatively related to larval nutrition, which is consistent with the idea that organisms can buffer the effects of nutritional constraints by changing allocation to the different fitness components (Stearns 1992). Although this study focused on nitrogen, long considered the key limiting nutrient for herbivores (Mattson 1980, Scriber & Slansky 1981; White 1984; Slansky & Rodriguez 1987; Bernays 1998; Awmack & Leather 2002), other nutrients, like phosphorus, might also influence herbivorous fitness (e.g. Huberty & Denno 2006; Apple et al. 2009). Thus, further progress on understanding the

link between larval nutrition with adult fitness may benefit from empirical studies considering latent effects when multiple nutrients are limited.

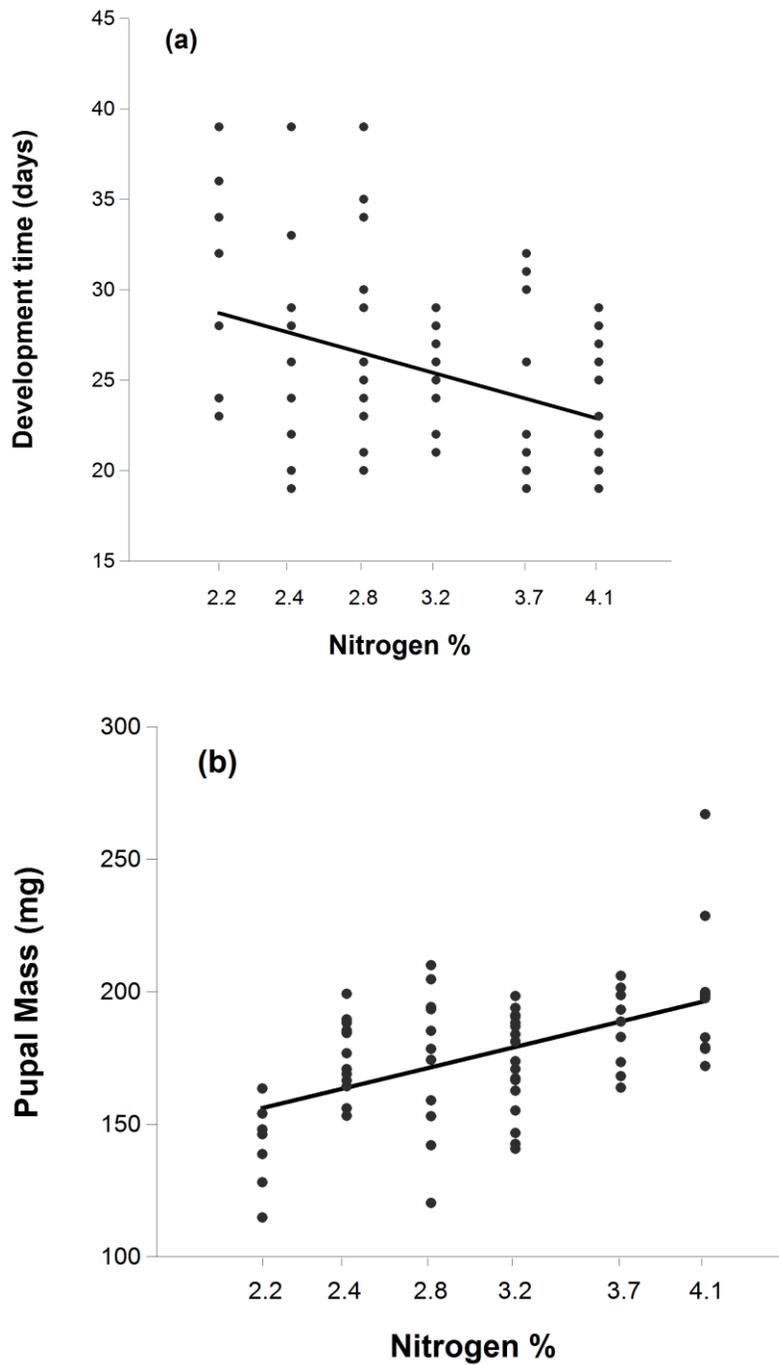


Figure 2.1. Effect of manipulating nitrogen concentration in the larval semisynthetic diet of male *P. rapae* butterflies on developmental traits: a) Development time (days from egg hatching to pupation). b) Pupal size (mass at 3rd day after pupation).

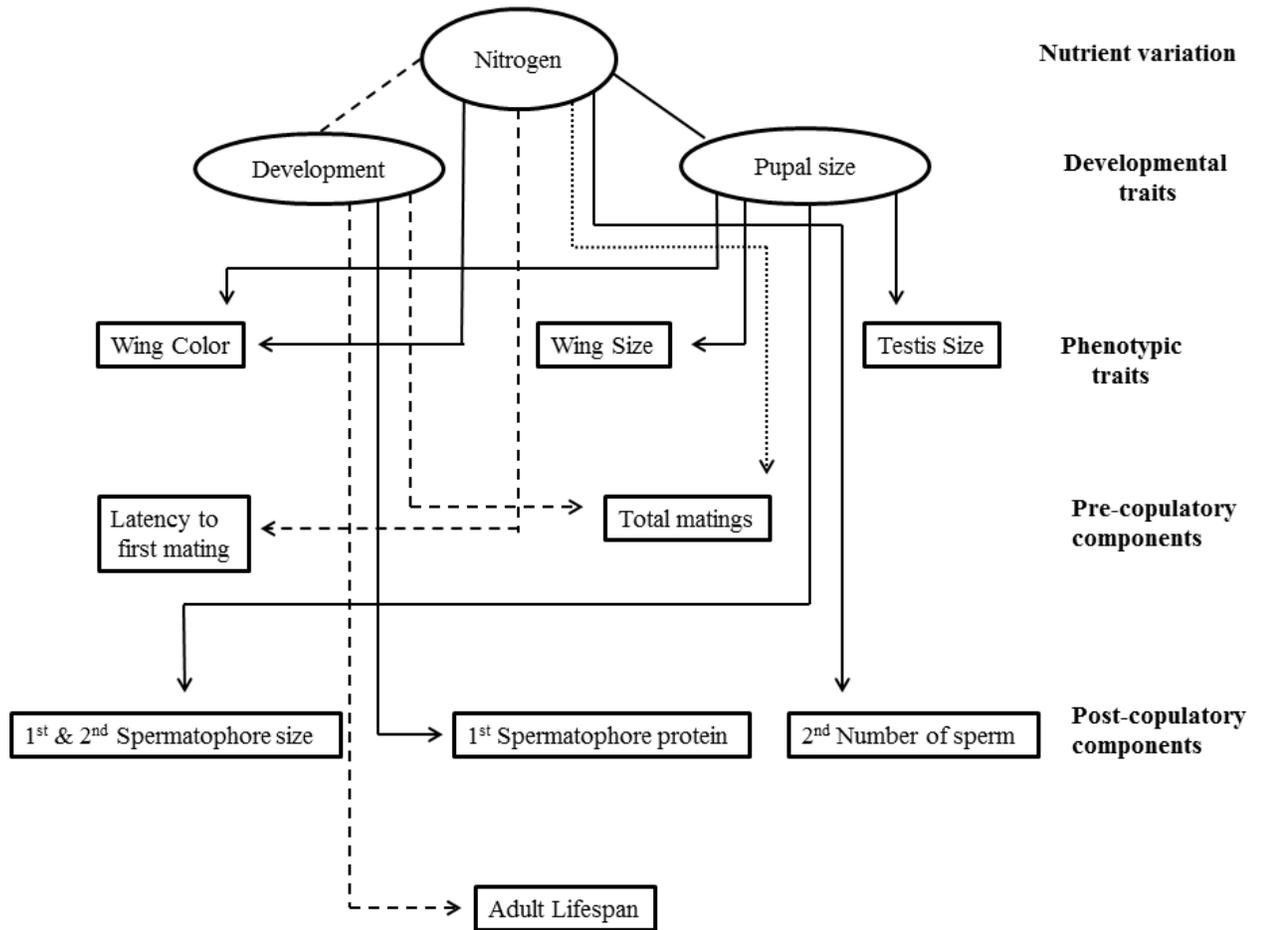


Figure 2.2. Summary of how dietary nitrogen during the larval stage of *P. rapae* males influenced adult phenotypic traits and different components of adult fitness both through and independent of development time and pupal size. Line types indicate significant positive (—), negative (---) or quadratic (.....) relationships.

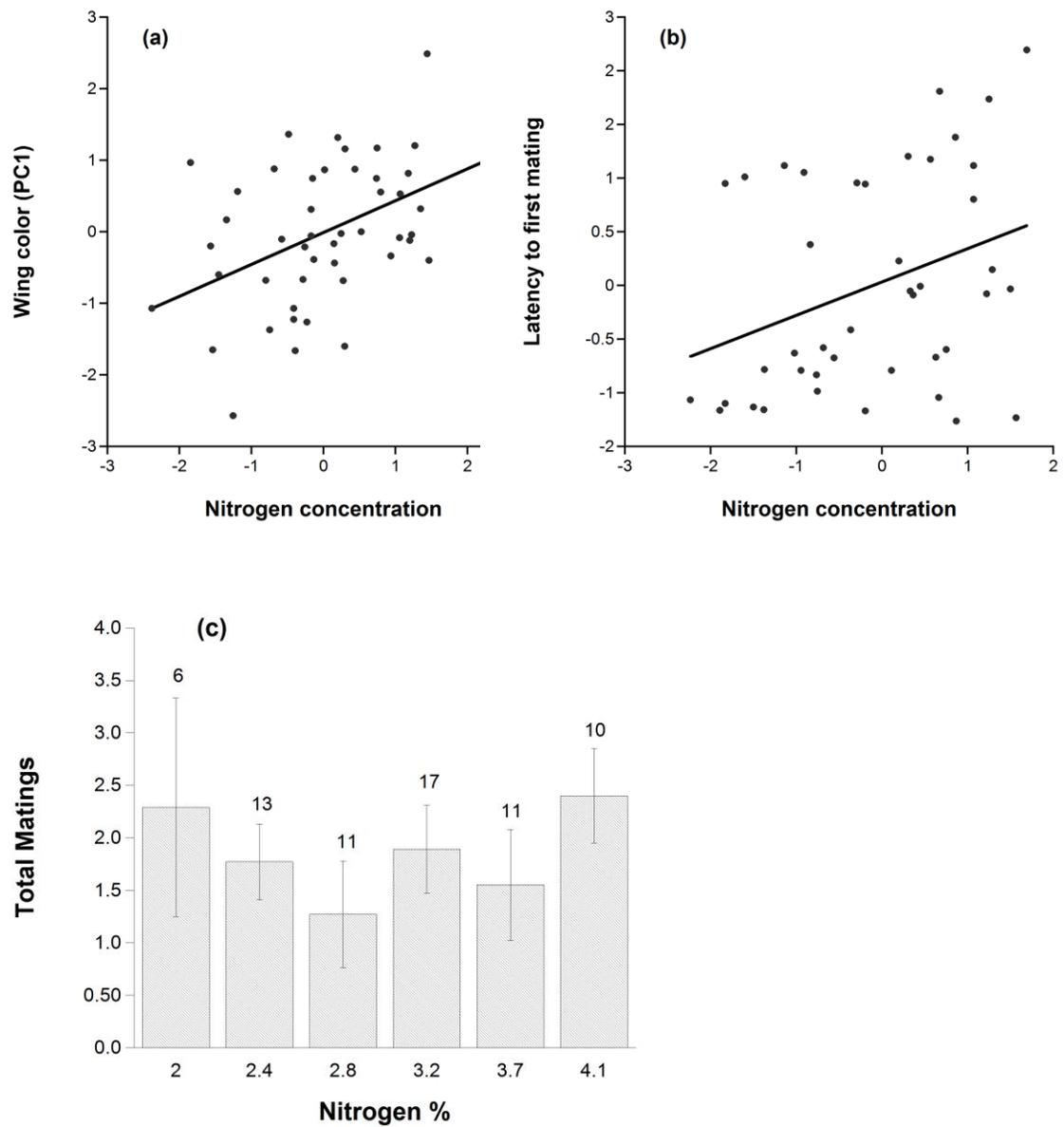


Figure 2.3. Effects of dietary nitrogen on different fitness-related traits of *P. rapae* males. a) Partial regression plot showing the effect of nitrogen, independent of development time and pupal size, on wing color (first principal component explaining 99% of variation). b) Partial regression plot showing independent effect of nitrogen on latency to first mating. c) Quadratic effect of nitrogen on mating success achieved by each male. Bars represent mean ($\pm 1SE$) number of matings for males reared on each nitrogen level. Numbers at the top of bars represent sample size.

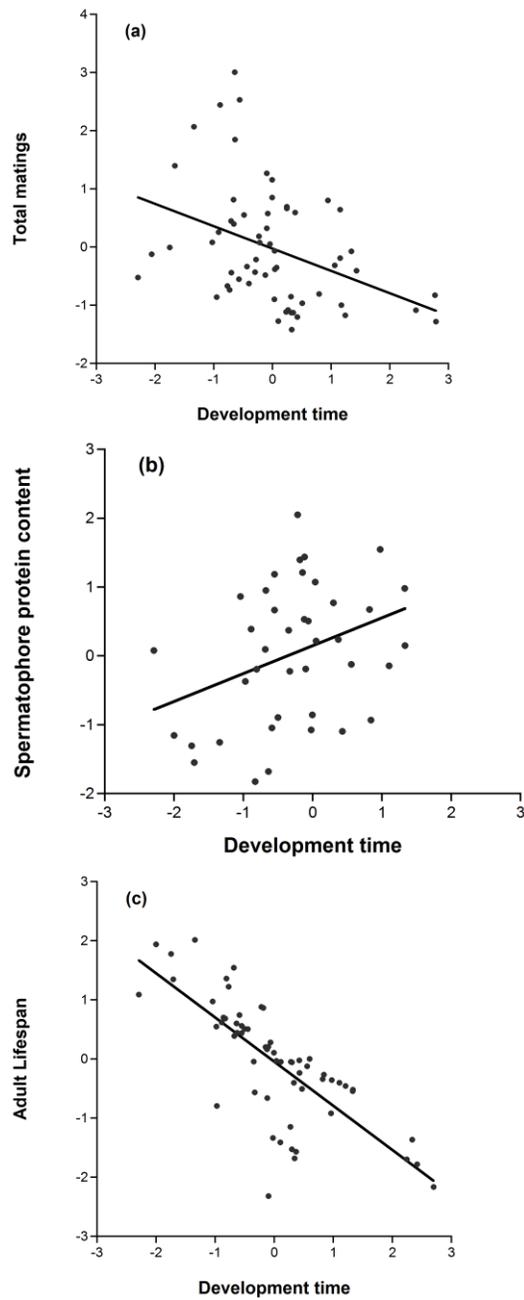


Figure 2.4. Partial regression plots illustrating the independent effect of development time (days from egg hatching to pupation) on male adult fitness related traits in *P. rapae*. a) Effect of development time on total number of matings b) Effect of development time on first spermatophore protein content (percent of total spermatophore wet mass) c) Effect of development time on adult lifespan.

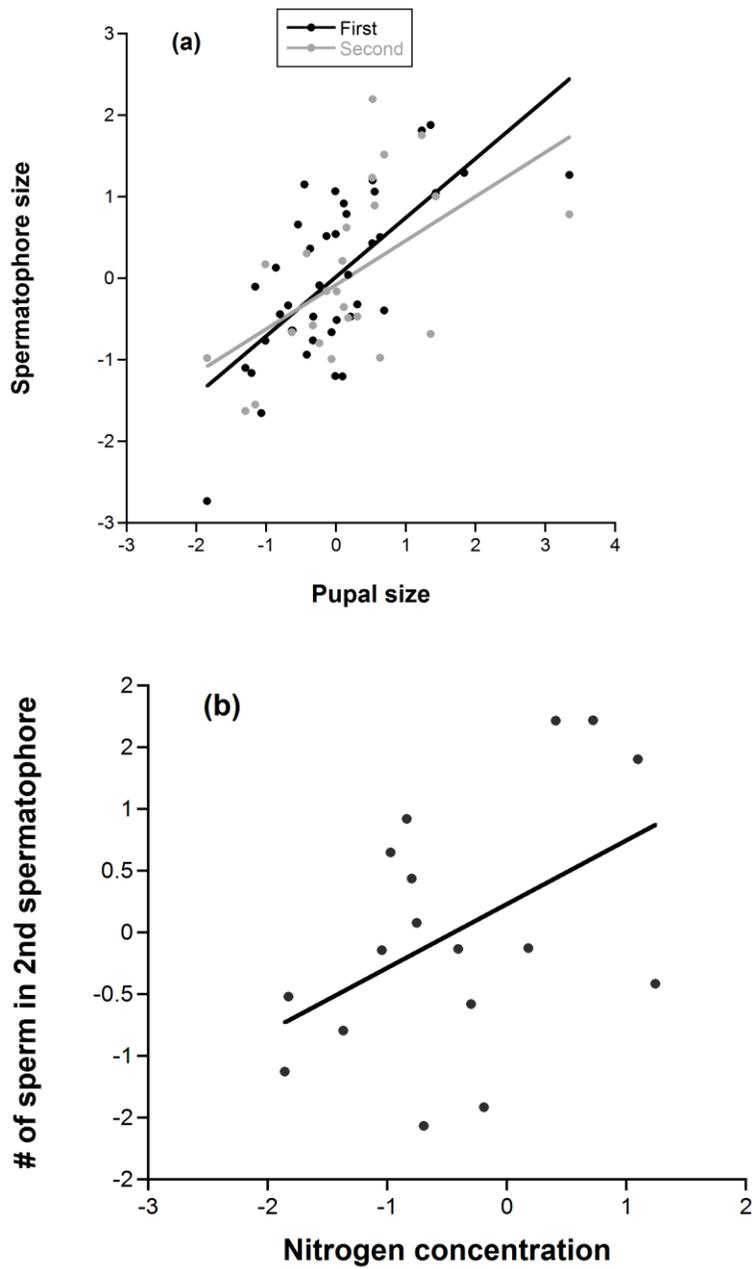


Fig. 2.5. Partial regression plots showing independent effect a) of pupal size (mass at 3rd day of pupation) on size of first and second spermatophore produced by *P. rapae* males and b) dietary nitrogen on number of sperm contained within second spermatophore.

Chapter 3: Sex-specific response to nutrient limitation and its effects on female mating success in a gift-giving butterfly

Abstract Animals with complex life cycles respond to early food limitation by altering the way resources are allocated in the adult stage. Response to food limitation should differ between males and females, especially in organisms whose mating systems include nutritional nuptial gifts. In these organisms, males are predicted to keep their allocation to reproduction (sperm and nuptial gift production) constant, while females are predicted to sacrifice allocation to reproduction (egg production) since they can compensate by acquiring nuptial gifts when mating. In this study, we investigated how dietary nitrogen limitation during the larval stage affects sex-specific resource allocation in *Pieris rapae* butterflies. Also, we tested whether nutrient-limited females increased nuptial gift acquisition as a way to compensate for low allocation to reproduction. We found that as predicted females, but not males, sacrifice allocation to reproduction when larval dietary nitrogen is limited. However, females were unable to compensate for this low reproductive allocation by increasing their mating rate to acquire additional gifts. Females reared on low nitrogen diets also reduced wing coloration, a potential signal of female fecundity status. We suggest that female mating frequency is constrained by male mate choice based on females' wing coloration. This study provides new insights into how larval dietary nitrogen, a

key nutritional resource for all herbivores, alters male and female allocation to reproduction as well as to ornamentation.

Introduction

Differences in resource availability during early life history stages can have major consequences for adult fitness. In holometabolous insects, decisions about how resources are allocated to adult structures must occur before or during metamorphosis. Previous studies have shown that differences in larval food quality or quantity can alter patterns of adult resource allocation, morphology and reproductive success in several taxa (e.g. Moczek 1998; Boggs and Freeman 2005; Bauerfield and Fischer 2005; Jannot et al. 2007; Boggs 2009).

However, effects of early food limitation are also predicted to differ between the sexes (Morehouse et al. 2010). Although individuals of both sexes should respond to nutrient limitation in ways that maximize their lifetime fitness (Collins 1980), males and females have distinct nutritional requirements based on their divergent reproductive roles. To date, only a few studies have examined sex-specific responses to early food limitation (but see Karlsson et al. 1997; Clarebrough et al. 2000; Hunt et al. 2004; Gwynne 2004; Boggs and Freeman 2005; Magwere et al. 2004; Maklakov et al. 2008; Stillwell and Davidowitz 2010).

Such sex-specific responses may be especially marked in organisms whose mating systems include nuptial gifts. Many insect males provide females with nuptial gifts during mating (reviewed by Boggs 1995; Vahed 1998; Gwynne 2008;

Lewis and South 2012). These gifts can be quite costly; male spermatophore gifts can represent up to 15% of the male's total body mass in some butterflies (Svärd and Wiklund 1989), and a single spermatophore produced by males of the butterfly *Pieris napi* contains the nitrogen equivalent of ~70 eggs (Karlsson 1998). Females in many taxa have been shown to use male-derived nutrients for somatic maintenance and to support egg production (reviewed by Boggs 1995). Female reliance on male gifts is altered by food limitation: when kept on low-protein diets, females of the cockroach *Blattella germanica* compensated with greater reliance on male-derived proteins (Mullins et al. 1992). Furthermore, male gifts are likely to be particularly important in organisms that lack adult feeding, since in this case female reproductive output must be fueled either by resources acquired from larval feeding or those obtained from male gifts. Thus, because nuptial gifts provide a link between male and female resource budgets, they have the potential to alter patterns of resource allocation by each sex.

Previous studies in lepidopterans suggest that patterns of adult resource allocation also depend on mating systems (Kaitala and Wiklund 1994; Bissondath and Wiklund 1995; Karlsson 1995; Karlsson 1996). In highly polyandrous mating systems, where females can potentially receive multiple nuptial gifts, food-limited females are predicted to reduce allocation of their own resources to reproduction because they could compensate later by acquiring several nuptial gifts (Leimar et al. 1994). However, under polyandry males are expected to maintain their allocation to reproductive structures at a relatively constant level even under food

limitation (Leimar et al. 1994). In monandrous species, where females will receive at most a single nuptial gift, females rather than males are expected to maintain their allocation to reproduction under food limitation (Karlsson et al. 1997). For polyandrous butterflies, Leimar et al. (1994) and Karlsson et al. (1997) predicted that food-limited females should increase their mating rates to acquire more nuptial gifts. However, empirical studies have yielded mixed support for these predictions (Leimar et al. 1994; Karlsson 1997; Bergström and Wiklund 2002; Boggs and Freeman 2005).

Availability of dietary nitrogen also impacts patterns of adult resource allocation. The reproductive processes of both female oogenesis and male gift production carry high protein requirements (Boggs 1995; Wheeler 1996). Herbivores are particularly susceptible to nitrogen limitation (Slansky and Rodriguez 1987; Mattson 1980) as plants typically contain considerably less nitrogen than animal tissue (Elser et al. 2000). Most previous studies of adult resource allocation in insect herbivores have manipulated food availability by using different plant species or restricting plant quantity (Leimar et al. 1994; Karlsson 1997; Boggs and Freeman 2005). However, these gross manipulations fail to target specific nutrients required to support reproductive activities of both sexes. Direct manipulations of dietary nitrogen, a key limited nutrient for most herbivores, might provide greater insight into the relationship between larval nutrient limitation and adult allocation patterns.

Larval food availability can impact allocation not just to primary reproductive structures such as ovaries, testes and male reproductive glands, but also to secondary sexual traits such as ornaments, weaponry, and body size. While costly traits are generally expected to decrease with food limitation (Andersson 1986; Cotton et al. 2004; Bonduriansky 2007), if food-limited females rely on access to male gifts acquired through mating, they should maintain or increase their allocation to ornaments or other sexual traits associated with mating success. For example, in the katydid *Kawanaphila nartee*, food-limited females fight for access to male nuptial gifts, and larger body size provides females with a mating advantage (Gwynne 2001). Under larval food limitation, *K. nartee* females maintained their body size to a greater extent than did males, while other traits showed a similar decrease across both sexes (Gwynne 2004). Thus, it is important to examine how early nutrition affects allocation not just to primary reproductive structures, but also to secondary sexual traits such as body size and ornaments.

In this study, we manipulated larval dietary nitrogen in a polyandrous and herbivorous butterfly, *Pieris rapae*, to examine how resource availability influences sex-specific allocation to traits associated with reproduction and mating success. We compared male vs. female allocation to wing, abdomen and thorax size, as well as to wing coloration, a trait known in this species to influence mating success of both sexes (Obara et al. 2008; Morehouse and Rutowski 2010a). We predicted that nitrogen-limited males would maintain their allocation to primary reproductive structures (abdomen size), while female reproductive

allocation would decrease. We also predicted that nitrogen-limited females would maintain their allocation to wing coloration, because this might allow them to acquire more nuptial gifts. In addition, we monitored adult mating success under realistic conditions in large outdoor flight enclosures to test the hypothesis that nitrogen-limited females would increase their gift acquisition by mating more often.

Materials and methods

Dietary nitrogen manipulation

Nitrogen is a key and limiting nutrient in herbivores such as butterflies, and variation in the availability of this nutrient is expected to alter allocation to adult body parts and other traits affecting reproductive success. Host plants of *P. rapae* naturally vary in nitrogen content ranging from 1.9% to 4.8% nitrogen (Slansky and Feeny 1977; Morehouse and Rutowski 2010b). In this experiment we manipulated dietary nitrogen within this range using a semi-synthetic diet following methods developed by Morehouse and Rutowski (2010b). Briefly, we replaced vitamin-free casein, the primary source of protein in this diet, with equivalent amounts of microcrystalline cellulose (FMC Biopolymer, Philadelphia, PA, USA), a non-nutritive and nontoxic filler. This allowed us to alter nitrogen concentration while leaving all other diet components constant. Semi-synthetic diets were prepared containing nitrogen percentages similar to those found in the natural diet of *P. rapae*; these included one “standard” diet (3.5%) and seven

treatment diets containing either 2.0, 2.4, 2.8, 3.2, 3.7, 4.1 or 4.4% nitrogen by dry mass. All larvae were reared on the standard diet (3.5% nitrogen) from hatching to 10 d (late 2nd- early 3rd instar), when they were randomly allocated to receive one of the seven diets until pupation. A group of control males was continuously reared on the standard diet from hatching until pupation.

Experimental individuals

All butterflies in this experiment were the F1 offspring of 9 singly-mated females that had been reared on standard diet from commercially obtained eggs (Carolina Biological Supply Company, Burlington, NC). To examine how larval diet affects mating success, we needed all the adults to emerge at approximately the same time, despite being reared on different diets. Because decreasing dietary nitrogen results in increased larval development times (Morehouse and Rutowski 2010b; Tigreros 2012) we synchronized adult emergence by first starting individuals assigned to the lowest nitrogen diets. To avoid confounding diet effects with any potential family or maternal effects, offspring from ≥ 3 different females were assigned to each diet treatment, and each female contributed eggs to ≥ 2 diet treatments; also, eggs from the first batch laid by each female were assigned to several different (low and high nitrogen) diets.

Individuals were kept from egg to pupation in individual 47 mm petri dishes (Fisher Scientific, Houston, Texas) stored in an incubator with an 18L: 6D photoperiod and 25°C: 20°C at 60% relative humidity. Each larva was provided

food *ad libitum*, consisting of a cube of semi-synthetic diet that was changed every 4 d. At emergence, some experimental males and females were frozen for later measurement of allocation to adult body structures. Mating trials (described below) were conducted with a subset of experimental females and the control males (reared continuously on 3.5% nitrogen); at emergence, these adults were fed with a 25% honey solution and then stored at $\sim 8^{\circ}\text{C}$ until the mating trials.

Measuring developmental traits

When resources are limited, individuals may prolong development times to acquire more resources; alternatively, they may show fixed development times and transition into adults at a smaller size (Nylin and Gotthard 1998). We examined dietary nitrogen effects on male and female development time (days from egg hatching to pupation) and pupal size (wet mass measured three days after pupation to $\pm 0.01\text{mg}$ accuracy).

Measuring allocation to adult body structures

We measured adult abdomen size, which for newly emerged butterflies is a good estimate of reproductive investment in both sexes (Boggs 1981). We also measured wing and thorax size; although these traits do influence adult performance (e.g. flight capability), they do not directly influence reproductive output. To measure wing size we photographically measured the right forewing area using ImageJ 1.45. Wing mass was estimated from area using a conversion

factor previously calculated from preliminary data (male forewing mass in mg = $0.10 + 0.73(\text{wing area in mm}^2)$, $n=14$, $r^2 = 0.5$ $p=0.005$; female forewing mass in mg = $0.25 + 0.92(\text{wing area})$, $n = 29$, $r^2 = 0.8$ $p < 0.0005$). Thorax and abdomen size were measured as dry mass measured to ± 0.01 mg accuracy.

We defined allocation as the proportion of total body mass represented by each of these body structures, calculated by dividing by the total adult dry mass for each individual.

Measuring wing coloration

In *P. rapae* butterflies, wing coloration has been shown to affect mating success for both sexes. Females choose mates based on the coloration of the male's dorsal forewing (Morehouse and Rutowski 2010a), while males find females based on the coloration of the ventral side of the female's hindwing (Obara and Majerus 2000; Obara et al. 2008). Thus for both sexes wing coloration is a signal that depends on the deposition of pterins, which are nitrogen-rich pigments that absorb ultraviolet wavelengths and increase reflectance in the visible spectrum (Morehouse et al. 2007).

In this study, we used reflectance spectrometry to measure pterin-based wing coloration according to Morehouse and Rutowski (2010a) protocols. Briefly, we used a JAZ spectrophotometer (Ocean Optics, Winter Park, Florida) to measure the spectral reflectance of a 2 mm diameter circle on the dorsal left forewing for males and on the ventral left hindwing for females. This spot was located between wing

veins CuA1 and 1A, close to the wing margin, to avoid the melanic spots and scattered melanic scales present in other portions of the wing. From the reflectance spectra measured in each individual, we calculated reflectance in the ultraviolet range ($R_{300-375}$) as well as reflectance in the visible range ($R_{550-650}$) (Morehouse and Rutowski 2010a).

Measuring female mating success

To examine how dietary nitrogen affects female mating frequency, we conducted six replicate mating trials in large outdoor enclosures. Each mating trial consisted of seven females, one from each different diet treatment; these seven females were placed together with 14 control males (reared on the 3.5% nitrogen diet). It was necessary to measure mating success on a different set of females from those used to measure wing coloration, because free-flying butterflies often lose or damage wing scales during courtship.

We set up a male-biased sex ratio to represent conditions found at the beginning of the mating season in natural populations (Shapiro 1970). Each group of seven females and 14 males was kept in spacious mating cages (Playhut EZ Outdoor portable screen room, 3 m L x 3 m W x 2.1 m H) placed outdoors. Prior to their release, females were individually marked by placing four colored dots (using Sharpie Extra Fine markers) near the base of the forewing on the costal margin area. These marks were ~0.8 mm diameter, and microscopic examination confirmed that they did not damage wing scales. Butterflies were left in the

outdoor cages for 5 d from 0700 to 1600 h and kept indoors overnight. Each mating cage was also provided with oviposition substrate (*Brassica oleracea* Linne) and wet sponges. After 5 d, all experimental females were frozen for later determination of their mating frequency by dissecting the bursa copulatrix and counting the number of spermatophore remnants (Drummond 1984).

Statistical analysis

To test for sex-specific effects of dietary nitrogen on adult developmental traits, morphology, and ornaments, we tested for homogeneity of slopes in an ANCOVA model with sex as a fixed factor and dietary nitrogen percentage as a covariate. A significant interaction between sex and diet indicated a sex-specific effect of larval nitrogen limitation. When no significant interaction was detected, the model was re-run without the interaction term to determine the main effects of sex and diet. All these analysis were performed using SPSS V18. Descriptive statistics are reported as means \pm 1 SE.

To examine how dietary nitrogen affected female mating probability, we used a generalized linear mixed model with a binomial response (mated/not mated) and a logit link function (Proc GLIMMIX in SAS ver. 9.2; The SAS Institute; Cary, NC, USA). To control for possible cage and family effects, we included these as random factors, with diet as the explanatory continuous variable.

Results

Larval diet effects on male and female development

We found no sex differences in how *P. rapae* individuals responded to changes in larval dietary nitrogen in terms of either development time or pupal mass (Fig. 3.1; ANCOVA test of sex x diet interaction on development time: $F_{1,90} = 1.21$, $P = 0.27$, on pupal mass: $F_{1,92} = 1.13$, $P = 0.29$). While males and females did not differ in their development times (sex effect $F_{1,91} = 0.59$, $P = 0.44$), males were significantly larger than females ($F_{1,93} = 17.72$, $P < 0.0005$) with males ($N = 61$) having an average pupal mass of 173.40 ± 3.45 mg compared to an average pupal mass for females ($N = 31$) of 150.42 ± 4.32 mg. Overall, increased dietary nitrogen led to significantly shorter development times (Figure 1A; diet effect $F_{1,91} = 57.64$, $P < 0.0005$; regression slope $b = -2.71 \pm 0.42$) as well as significantly higher pupal mass ($F_{1,93} = 25.75$, $P < 0.0005$; $b = 11.95 \pm 2.69$).

Larval diet effects on male and female resource allocation

There was no sex-specific effect of larval dietary nitrogen on allocation to wing size in *P. rapae* butterflies (Fig 3.2A; sex x diet interaction: $F_{1,71} = 1.96$, $P = 0.16$). Overall, allocation to wings was similar between males and females (sex effect: $F_{1,72} = 1.89$, $P = 0.17$), and wing allocation significantly increased with reduced dietary nitrogen in both sexes (diet effect: $F_{1,72} = 12.22$, $P = 0.001$; regression slope $b = -0.004 \pm 0.001$). Similarly, there was no interaction between sex and diet for thorax size (Fig 3.2B; sex x diet interaction: $F_{1,71} = 0.74$, $P = 0.39$). Males showed significantly higher thorax allocation compared to females (sex effect:

$F_{1,72} = 23.64$, $P = 0.0005$), but this did not change in response to dietary nitrogen (diet effect: $F_{1,72} = 0.002$, $P = 0.96$).

In contrast, manipulating dietary nitrogen revealed a significant sex-specific effect on allocation to abdomen (Fig. 3.2C; sex x diet interaction: $F_{1,71} = 4.29$, $P = 0.04$). Females seem to allocate more to abdomen compared to males (Fig. 3.2C), but this declined with lower dietary nitrogen while males' allocation to abdomen remained constant (for males, regression slope $b = 0.001 \pm 0.008$, $N = 61$; for females, $b = 0.029 \pm 0.009$, $N = 31$).

Larval diet effects on male and female wing coloration

Dietary nitrogen had a similar effect on males and females for both measures of wing coloration (Fig 3.3; sex x diet interaction for UV reflectance: $F_{1,92} = 0.21$, $P = 0.64$; for visible range reflectance: $F_{1,92} = 0.23$, $P = 0.63$). In addition, male dorsal forewings were less reflective in the UV range than female ventral hindwings (sex effect $F_{1,93} = 97.97$, $P < 0.0005$) and more reflective in the visual range ($F_{1,93} = 1385.21$, $P < 0.0005$) Also, we found that dietary nitrogen affected wing coloration of *P. rapae* males and females, with individuals reared on the highest nitrogen diets being more colorful than those in the lowest nitrogen diets; with increased dietary nitrogen, UV reflectance declined ($F_{1,93} = 5.41$, $P = 0.02$; female regression slope $b = -0.211 \pm 0.15$ male $b = -0.217 \pm 0.13$), and reflectance in the visible spectrum increased ($F_{1,93} = 7.18$, $P = 0.009$; female $b = 1.078 \pm 0.61$, male $b = 1.21 \pm 0.57$).

Larval diet effects on female mating success

To examine how dietary nitrogen affects female mating probability, we conducted outdoor mating trials under realistic conditions; in each of 6 replicate trials, 7 females each reared on different diet treatments were allowed to interact for 5 d with 14 control males. During these trials 72.5% of the 42 females mated once, while only one female mated twice, and 25% of the females failed to mate. Females that had been reared on higher nitrogen diets were significantly more likely to mate (Figure 3.4 and Table 1; $t = 2.2$, $df = 34$, $P = 0.033$). Also, in these trials at least 64% of the 84 males did not mate at all, since across all trials we observed only 30 matings.

Discussion

Studies at the interface between sexual selection and nutritional ecology are needed to better understand how ecological context can influence this key evolutionary process. Animals with complex life cycles are known to respond to nutrient limitation during juvenile stages by altering patterns of adult resource allocation and morphology (e.g. Moczek 1998; Boggs and Freeman 2005; Bauerfield and Fischer 2005; Jannot et al. 2007; Boggs 2009). Although males and females are predicted to respond differently to early nutrient limitation (Morehouse et al. 2010), few studies have examined such sex-specific responses. In this study, we experimentally manipulated nitrogen availability for larval *Pieris*

rapae butterflies, an herbivore known to be nitrogen-limited (Morehouse and Rutowski 2010b), highly polyandrous (Svärd and Wiklund 1989) and in which males provide females with resource-rich spermatophore gifts (Svard and Wiklund 1989). Our results revealed that early nutrient limitation altered adult resource allocation for both sexes, but exerted very different effects on males and females.

As predicted for polyandrous gift-giving butterflies (Leimar et al. 1994), we found that nitrogen-limited *P. rapae* males maintained their allocation to reproductive structures measured as relative weight of the abdomen, which is where males' spermatophore-producing glands are located. In contrast, *P. rapae* females that were reared on low-nitrogen diets reduced their allocation to abdomen (Figure 5). On the other hand, both sexes showed similar responses to nitrogen limitation for adult traits not directly connected to reproduction, such as wings and thorax. These results suggest that stronger selection is acting on males to allocate their larval-derived nitrogen to spermatophore production than is acting on females to allocate such resources to egg production.

In insects, allocation to spermatophore production can increase male reproductive success across several selection episodes (Lewis and South 2012). Producing larger spermatophores is advantageous because this increases female fecundity (e.g. Butlin et al. 1987; Watanabe and Ando 1993). Larger spermatophores also reduce the likelihood that a female will remate (e.g. Sugawara 1979; Kandori and Ohsaki 1996), and increase male paternity share

when females do remate (e.g. LaMunyon and Eisner 1994; Wedell and Cook 1998). Additionally, the rate at which males can produce spermatophores has been suggested to limit male mating rates in other insects (Fedina and Lewis 2006).

Wing coloration in both sexes of *P. rapae* is based on nitrogen-rich pterin pigments (Morehouse et al. 2007), and we found similar responses to limited dietary nitrogen between males and females: for both sexes, individuals reared on low nitrogen diets showed less colorful wings (i.e. increased UV reflectance and reduced reflectance in the visible range). Previous work has demonstrated that male dorsal forewing coloration is a sexually selected ornament in *P. rapae* males (Morehouse and Rutowski 2010a), and female ventral hindwing coloration has been shown to influence female mating success (Obara and Majerus 2000; Obara et al. 2008). Our results agree with other work by Morehouse (2009), which showed that males and females reared on high nitrogen diets developed more colorful wings than those on low nitrogen diets. Thus, pterin-based wing coloration might provide information about the quality of an individual's nutritional environment to potential mates. Previous studies in the Pieridae have proposed that male wing coloration provides females with an honest signal of spermatophore quality (Kemp and Rutowski 2007; Morehouse 2009). We found that females reared on low nitrogen diets not only have reduced wing coloration, but also lower abdomen allocation, suggesting that female wing coloration could also be used as a reliable signal of female quality.

Contrary to predictions (Leimar et al. 1994) our mating trial results showed that *P. rapae* females reared on lower nitrogen diets had significant reductions in their mating success. In contrast, male mating success has been shown to be only weakly affected by larval nitrogen limitation (Tigreros 2012). Results of the current study indicate that, at least under these experimental conditions, females were unable to compensate for their low investment into reproductive structures by acquiring more nuptial gifts. Other studies have shown that butterflies receiving small nuptial gifts or deprived of food as adults increase their mating rates and thereby are able to accumulate additional male gifts (Kaitala and Wiklund 1994; Boggs and Ross 1993). However, our results, together with Bergström and Wiklund's (2002) study in *P. napi*, indicate that females cannot compensate by acquiring extra nuptial gifts when they experience nutrient limitation during the larval stage.

There are several possible explanations for this decreased mating frequency of low nitrogen females. One possible explanation is that female mating frequency is constrained by male mate choice. Male mate choice is predicted to evolve when males invest substantially in reproduction (Bonduriansky 2001; Edward and Chapman 2011), as occurs in *P. rapae* and other gift-giving butterflies. Thus, *P. rapae* males would benefit from discriminating against low nitrogen females (whose lower abdomen allocations suggest they are less fecund), perhaps by using female wing coloration as indicator of female quality. Another possibility is that *P. rapae* females reared on

low nitrogen diets may have reduced receptivity to mate. Studies in different insect taxa (e.g. cockroaches, true bugs and fleas) indicate that dietary constraints can decrease females' receptivity to mate (reviewed in Ringo 1996). Also, dietary constraints may affect other aspects of female phenotype and performance, for example reducing female activity and thus lowering encounter rates. Additional studies measuring male and female activity and courtship interactions would be useful in differentiating among these possible explanations for the observed decline in mating frequency of *P. rapae* females reared on low nitrogen diets.

As *P. rapae* females that are nitrogen-deprived apparently do not compensate by mating more often to acquire more nuptial gifts, why do females sacrifice their allocation to reproductive structures instead of keeping this constant as males do? We offer two possible explanations for this dilemma. First, when nitrogen is limited, the total resource pool acquired by females is about 17.6% less than that of males (Fig. 5). Thus, greater female constraints may require tradeoffs between allocation to reproduction vs. other traits necessary for survival. Second, females may be able to acquire resources through adult feeding as adult females in some butterflies may use nectar not only as an energy source, but also to acquire resources for egg production (e.g. O'Brien et al. 2004).

In summary this study shows that when larval dietary nitrogen is limited, females but not males sacrifice their investment into reproduction in *P. rapae*, a gift-giving, polyandrous butterfly. Female mating success is negatively affected by poor larval nutrition, and thus females are unable to compensate for low

reproductive allocation by increasing mating rates to acquire additional gifts. This study broadens our understanding of the link between nutritional ecology and sexual selection and provides new insights into how larval dietary nitrogen, a key nutritional resource for all herbivores, alters male and female allocation to primary and secondary sexual traits.

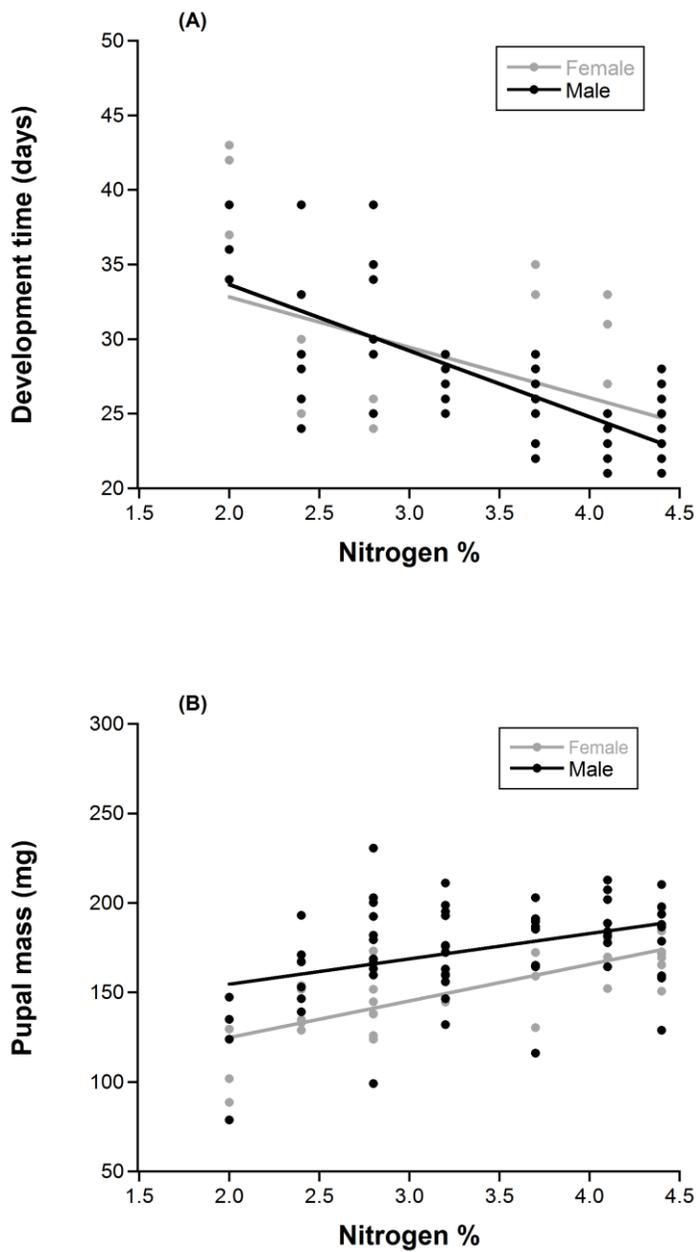


Figure 3.1. Effect of manipulating larval dietary nitrogen on developmental traits of *P. rapae* butterflies: A) Development time (days from egg hatching to pupation). B) Pupal mass (measured at 3rd day after pupation).

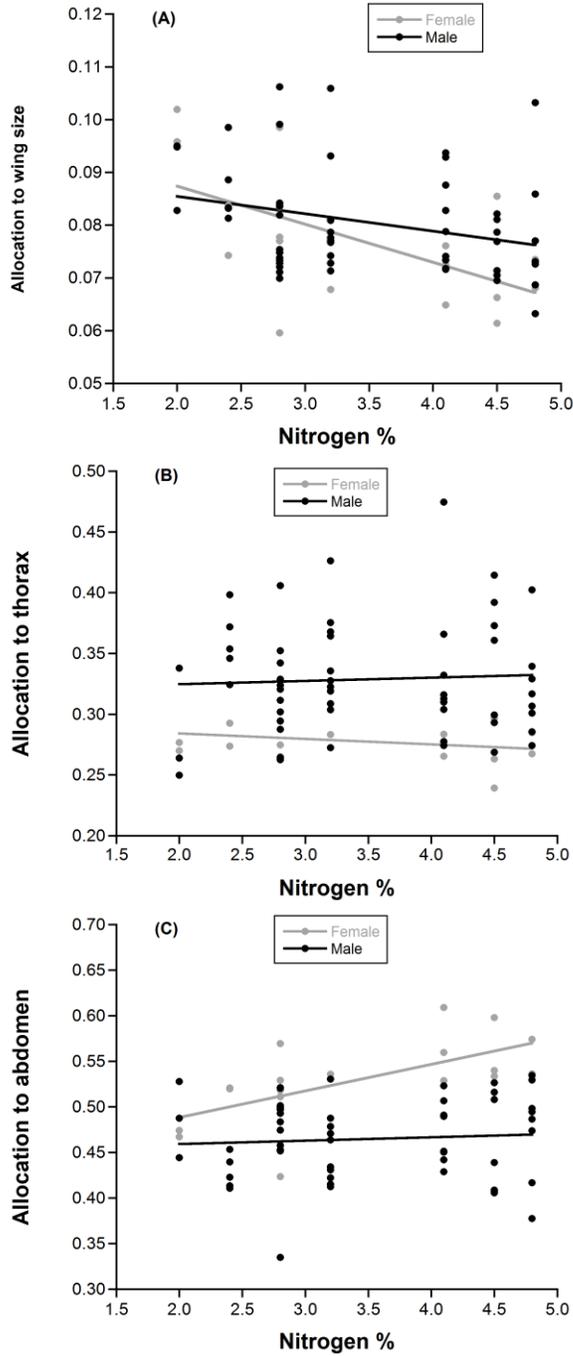


Figure 3.2. Effects of manipulating larval nitrogen on proportional allocation (dry mass/total body mass) to adult structures by male and female *P. rapae*: A) allocation to forewings B) allocation to thorax C) allocation to abdomen.

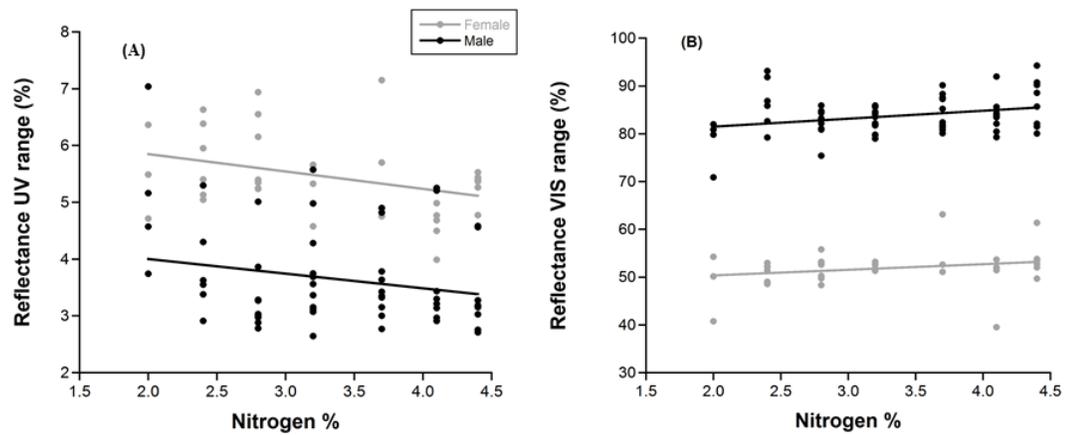


Figure 3.3. Effects of dietary nitrogen on pterin-based wing coloration of *P. rapae* butterflies (spectral reflectance measured on dorsal forewings for males and ventral forewings for females). A) Percent reflectance in the ultraviolet range (300 to 375 nm) and B) Percent reflectance in the visible range (550 to 650 nm).

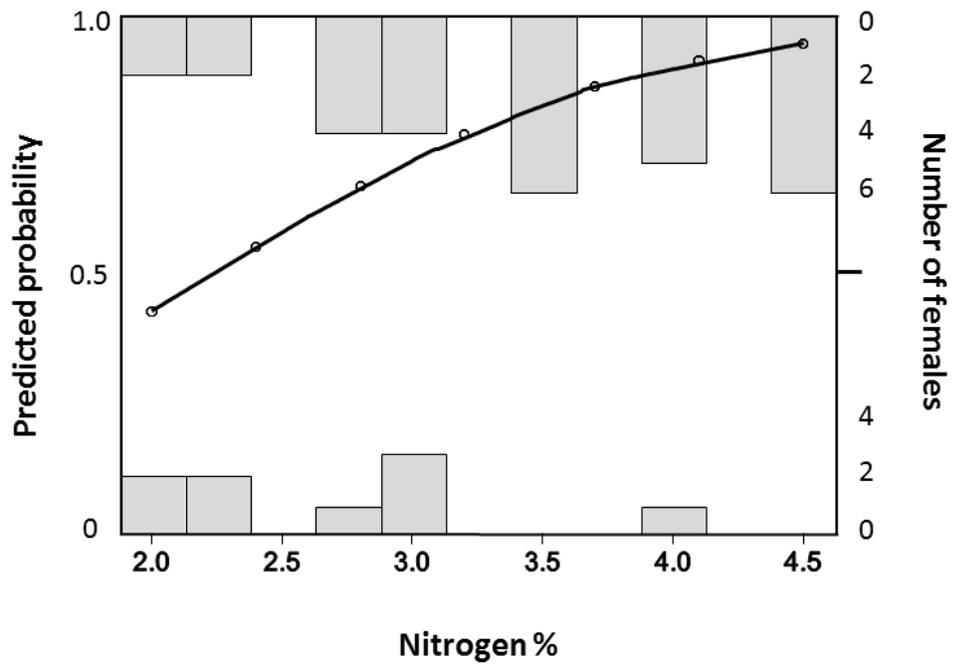


Figure 3.4. Effects of larval dietary nitrogen on the mating success of female *P. rapae* butterflies during 5 d mating trials conducted in large outdoor enclosures. Upper and lower histograms show observed frequencies of mated and unmated females, respectively, and the line shows the predicted probability of mating for females reared at each nitrogen level.

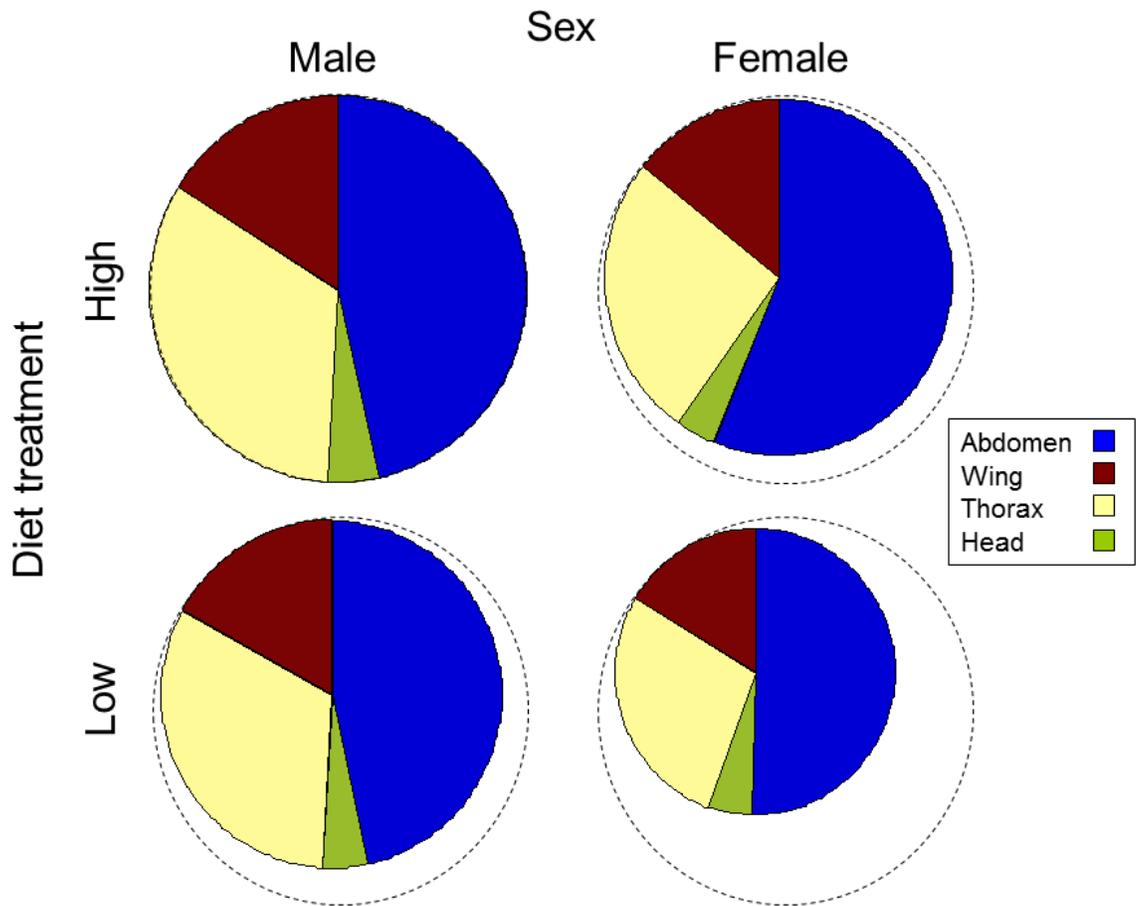


Figure 3.5. Summary of sex-specific resource allocation to adult structures when *P. rapae* butterflies were reared on either high nitrogen (above 4%) or low nitrogen (below 3%) semi-synthetic diet. For comparison, dotted lines show the size of the total resource pool acquired by males under high nitrogen availability.

Table 3.1. Output of a generalized linear mixed model to test for an effect of larval dietary nitrogen on the mating success of female *P. rapae* butterflies (to control for possible effects of cage and family, these were included as random factors).

Effect	Estimate	SE	df	P
Intercept	-6.31	2.69	34	0.025
Diet	1.69	0.76	34	0.033

Covariance parameter	Estimate	SE
Cage	0.68	1.13
Family	0	—

Chapter 4: Male mate choice favors more colorful females in the gift-giving cabbage butterfly

ABSTRACT

While the phenomenon of male mate choice has attracted considerable attention in the last two decades, whether this sexual selection mechanism could drive the evolution of female ornaments remains poorly understood. Here, we used experimental manipulation of female wing coloration to investigate male mate choice in *Pieris rapae*, a gift-giving butterfly. Further, we tested whether males' nutritional status influenced their mating preferences by subjecting larvae to short periods of starvation. We found that males preferentially approached and attempted to copulate with females having more colorful wings (higher pterin content), and that this preference was strongest in the low nutrition males. Additionally, a study of field-collected females revealed that pterin-based wing coloration was positively correlated with female egg load, which suggests such ornaments may honestly signal female quality. Pterin-based ornaments are widespread in nature, however their potential as honest signals in male mate choice remains mostly unexplored.

Introduction

Since Darwin (1874) first proposed sexual selection through mate choice as an important mechanism driving evolutionary change, females have typically been

considered to be the choosier sex as they select more highly ornamented males as mates. However, theoretical and empirical evidence gathered over the last decade challenges the traditional gender roles of choosy females and promiscuous males (Bonduriansky 2001; Clutton-Brock 2009). Male mating preferences are predicted to evolve when male mating investment is large, when female mate quality variance is high, or when mate search costs are low (Burley 1977; Parker 1983; Owens and Thompson 1994; Johnstone et al 1996; Kokko and Monaghan 2001). Indeed, male mate choice has now been well-documented in numerous taxa, including birds (e.g. Amundsen 2000; Forero et al. 2001; Sæther et al. 2001; Kraaijeveld et al. 2004), fish (e.g. Amundsen, T. & Forsgren 2001; Foote et al. 2004; Tudor and Morris 2009), lizards (e.g. Weiss 2006), mammals (e.g. Setchell and Wickings 2002; Preston et al. 2005) and insects (reviewed by Bonduriansky 2001).

Despite the growing recognition of male choice, it remains controversial whether such mate choice can select for female ornamentation (Bonduriansky 2001, 2009; Tobias et al. 2012). Although female ornaments are strikingly common in animals (e.g. Amundsen 2000; Lee 2007), the evolution of costly sexual displays in females has been considered unlikely due to the competing demands between ornaments and egg production (Fitzpatrick et al. 1995; Chenoweth et al. 2007; Morales, Velando & Torres 2009; but see Doutrelant et al. 2008; Simmons & Emlen 2008). Thus, the existence of female ornamentation has been viewed as a non-adaptive correlated response to selection on male ornaments (Lande 1980, Lande and Arnold 1983). Alternatively, female ornamentation may

evolve if it increases female's chance of gaining material benefits from males (Kokko and Johnstone 2002; Kraaijeveld et al. 2007). Males in many taxa provide females with a nuptial gift during mating (Lewis et al. 2011), and such gifts can increase a female's lifetime fecundity (Boggs 1995; Lewis and South 2012). Thus, in gift-giving species, female ornaments could evolve through direct selection to increase access to these material benefits. It is clear that the role of male choice in selecting for female ornaments deserves further study (Bonduriansky 2001, 2009, Nordeide et al. 2013), particularly in species whose mating systems include a nuptial gift.

Similarly to female mate choice, male mate choice is also expected to be variable (Bonduriansky 2001; Hardling and Kokko 2005; Servedio 2007). Empirical work has identified some factors that alter male choice such as male mating history (Byrne and Rice 2006; Nandy et al. 2012) and male phenotypic quality (Holveck et al. 2011). Additionally, theory posits that the cost-benefit balance of mate choice should vary according to condition (McNamara and Houston 1996). Thus, individuals in poor physiological condition are expected to be less choosy about mates than those in better condition (Hardling and Kokko 2005). However, to our knowledge no studies have empirically evaluated whether male preferences are altered by nutritional status. Nutritional constraints are quite common in nature and are known to impact many aspects of male physiology and life history (e.g. lifespan, reproductive investment) that in turn could influence the costs of mate choice. Thus, it is important to understand how nutrition affects

male mating preferences, since this can change the strength and direction of sexual selection (Cotton et al. 2006).

Butterflies are a highly appropriate group for investigating male mate choice and female ornaments. The striking color pattern variation in wings of both sexes has long been attributed to intersexual selection (Darwin 1874). Males provide females with nuptial gifts in the form of spermatophores that can represent up to 15% of the male's total body mass (Svärd and Wiklund 1989). These gifts provide females with material benefits, as male-derived nitrogen can be traced to female oocytes (Boggs and Gilbert 1979); a single *Pieris napi* spermatophore, for example, contains the nitrogen equivalent of ~70 eggs (Karlsson 1998). Lastly, previous studies investigating the early stages of courtship suggest the possibility of male choice (Rutowski 1982; Knuttel and Fiedler 2001; Ellers and Boggs 2003).

The goal of this study is to better understand the evolution of female ornamentation under male mate choice using experimental and correlational studies in a nuptial gift-giving butterfly, *Pieris rapae*. We conducted choice experiments with experimental manipulation of female wing coloration to investigate male mating preferences based on female ornaments. We also assessed variation in male preferences by altering male nutritional status during the larval stage. Finally, to determine whether males could potentially use female ornaments as a signal of fecundity, we examined the correlation between wing coloration and egg load in field-collected females.

Methods

Study species

In *P. rapae*, both males and females mate multiple times and males invest in reproduction, by donating a nuptial gift to the female during copulation. This nuptial gift consists of a protein-rich spermatophore (Bissoondath & Wiklund 1995), consisting of ~10% of the male body weight (Tigreros 2013). Receiving multiple spermatophores significantly increases female reproductive output (Watanabe & Ando 1993).

Female mate choice has been documented in *P. rapae*, with females preferring males with more colorful wings based on higher pterin content of their wing scales (Morehouse & Rutowski 2010). Pterins are nitrogen-rich pigments (Kayser 1985) that absorb ultraviolet wavelengths and increase reflectance in the visible spectrum (Morehouse et al. 2007). Females also have pterins deposited on their ventral hindwings, and in both males and females pterin concentration honestly signals nutritional conditions experienced during larval feeding (Morehouse 2009; Tigreros 2013).

Additionally, *P. rapae* visual sensitivity allows males to discriminate between the sexes based on wing coloration (Arikawa et al. 2005); previous work in *P. rapae rapae* (European subspecies) indicates that female wing coloration is used for sex recognition (Obara and Majerus 2000). Also, male preferences for female wings with high UV reflectance has been suggested in *P. rapae crucivora* (Japanese subspecies) (Obara et al. 2008).

Courtship behavior in *P. rapae* is typically characterized by a behavioral sequence described by Suzuki (1977): a *Mating approach*, when a male lands beside a resting female facing in the same direction, continuously fluttering his wings and catching the female's forewing with his legs; *Copulation attempt*, in which the male curves his abdomen dorsolaterally in attempt to couple with the female genitalia; *Successful copulation*, genital coupling that occurs after the male is accepted by the female; mating lasts about 2 hours. Mating approaches also occur while females are in flight; these are characterized by males following a flying female flying in a zig-zag pattern while positioned behind and slightly below her (Suzuki 1977). *P. rapae* females control the success of male mating attempts, as they can refuse males by flying away or by curving their abdomen upward (Suzuki 1977).

Measuring wing coloration and fecundity in field-caught females

In mid-July 2011, we collected 43 females from the field in the Boston area. Females were frozen immediately, wings were kept in glassine envelopes and abdomen were stored in 85% ethanol for later examination.

Female potential fecundity: Each female abdomen was dissected, and the total number of oocytes (including mature and immature eggs) was counted using a dissecting scope.

Measuring wing coloration: Previous studies in *P. rapae* (Obara and Majerus 2000; Obara et al. 2008) and other butterflies have shown that males respond to visual stimuli located on the ventral side of the female's hindwing. We used reflectance

spectrometry to measure wing coloration according to protocols developed by Morehouse and Rutowski (2010). We used a JAZ spectrophotometer (Ocean Optics, Winter Park, Florida) to measure the reflectance of a 2 mm diameter spot located at the margin of the ventral left hindwing along the central vein. Reflectance was measured across wavelengths from 300 to 650 nm, which covers the visual range of butterflies (Eguchi et al. 1982). For each of the 43 females, we calculated two parameters relevant to both pterin coloration and male vision (Arikawa et al. 2005; Morehouse and Rutowski 2010): average reflectance across the ultraviolet range ($R_{300-375}$) as well as average reflectance across the visible range ($R_{550-650}$). Principal component analysis was used to reduce these into a single principal component, PC1, that represents pterin-based wing coloration.

Determining body size and age: To control for possible confounding factors on the relationship between female fecundity and wing coloration, we estimated female body size and age. Adult size was estimated as hindwing length measured diagonally from the shoulder to the wing margin using calipers; hindwing length scales isometrically with body mass in butterflies (Frankino et al. 2005, 2007, Tigreros unpublished data). We estimated female age based on wing wear, assigning females to three categories, based on the condition of the margins, scales, and extent of breakage in the wings (Watt et al. 1977).

Male Choice and Female Coloration

Individuals for this experiment were the F1 progeny of field-caught females that were reared as larvae on organic cabbage (*Brassica oleracea*

convar. *capitata* var. *alba* L.) in climate-controlled incubators at 27°C: 20°C (16L: 8D, 60% humidity). Butterflies were reared from egg to adult in individual containers, and adults were fed a honey-water solution (1:3). All individuals participating in the mate choice trials were virgins, and were used within 72 hours of eclosion.

Manipulating female wing coloration

To experimentally test whether female wing coloration is a trait subject to sexual selection by male mate choice, we manipulated wings of live females to generate color variation within the natural range (Fig. 4.1). We randomly assigned females to two groups: pterin-extracted and control females. In the first group, we removed pterins—from the wings modifying methods described by Morehouse and Rutowski (2010). Here, pterins were extracted using an aqueous solution of 3.3 mM NaOH, which removed pterins without damaging wing scales or altering any other morphological characteristics of the wing (Rutowski et al. 2005). Live females were held gently with forceps and submerged for 10 s in 50% isopropanol, then in 3.3 M NaOH for 10 s, and finally returned to 50% isopropanol for 10 s. Control females were submerged twice for 10 s in 50% isopropanol, keeping wing pterins intact while controlling for any potential handling effects. Because this procedure occasionally caused injury if solutions wicked onto the body, we verified normal flying behavior in all individuals before they were used in the mating trials.

Manipulating male nutrition: In *P. rapae* larval nutrition has been shown to alter several life history traits (e.g. development time, body size, adult lifespan) that

impact male fitness (Tigreros 2013). To test whether males' mate preferences as adults change depending on their larval nutrition, we manipulated food availability during larval development. Preliminary experiments were used to determine a sublethal starvation regime strong enough to alter male condition (e.g. significant changes in development time and pupal size). Males randomly assigned to the high nutrition treatment (n=17) were fed cabbage *ad lib* throughout their larval development, while males assigned to the low nutrition treatment (n=16) were subjected to two separate starvation periods; they were initially starved for 4 h at day 11 after larval eclosion, (when they had reached late 3rd-early 4th instar), and then again starved for 24 h at day 13 (4th instar).

Mate choice trials: We conducted pair-choice trials by simultaneously presenting each male with one pterin-extracted female and one control female. Females within each pair were matched according to pupal mass (within 10%) and each was marked near the base of the forewing on the costal margin area with a tiny colored dot (Sharpie Extra Fine markers).

Each trio was placed in an outdoor cage (24 inch tall, 15 1/2 inch in diameter) where individuals could fly freely. Courtship behaviors were observed continuously for 20 min or until a copulation attempt occurred. We recorded the number of male mating approaches (defined above), as well as copulation attempts (defined above) that were directed toward each female. As soon as a copulation attempt occurred, the trios were separated and the trial was concluded (i.e. females were not allowed to mate).

Following this first mate choice trial, females were fed and after 3 h the same female pair was assigned to a second trial with a male that had been reared under the opposite nutrition treatment (starved or not); across female pairs, we randomized the presentation order of the two males.

Statistical analysis

To examine the relationship between female fecundity and hindwing coloration, we used ANCOVA with total number of eggs as the dependent variable and pterin-based wing coloration (PC1) as a predictor; we included age (as a categorical fixed factor) and hindwing size (continuous) as covariate. To investigate male mating preferences based on female ornaments we used logistic regression (SAS proc logistic) which modeled male response as proportion of each male's approaches that were directed towards the control female over the total number of approaches he made to both females. We first tested for the significance of the intercept with no predictor variables to estimate the overall male mating preference (approaches to control vs. pterin-extracted females). Then, we tested for an effect of male nutrition on his mating preferences by including male nutritional as predictor variable. Because the effects of testing a same pair of females with a high and a low condition male were not statistically significant we excluded this variable from the final model. SAS ver. 9.1 was used for data analysis. Descriptive statistics are reported as means \pm 1 SE.

Results

Wing coloration and fecundity in field-caught females

For 43 field-collected *P. rapae* females, UV and visible reflectance of ventral hindwings averaged 42.8 ± 0.9 % and 7.0 ± 0.3 % respectively. The first principal component captured 92% of the observed variation in female wing coloration and was positively correlated with visible reflectance (factor loading = 0.99) and negatively correlated with UV reflectance (factor loading = -0.13). We found that increased female coloration (in terms of pterin-based wing coloration, PC1) was positively associated with female egg load (Fig. 4.2; ANCOVA, $F_{1,36} = 7.9$, $P = 0.008$). However, female egg load (median = 193 oocytes, IQR=139) was not significantly affected by differences in either female age (estimated by wing wear; $F_{2,36} = 0.48$, $P = 0.66$) or body size (hindwing length: $F_{1,36} = 0.13$, $P = 0.7$).

Male Choice and Female Coloration

During the mate choice trials, *P. rapae* males approached both of the females at least once and up to 15 times (a median of 4 mating approaches). Males were more likely to direct a mating approach towards a control female rather than a pterin-extracted female (Fig 4.3 and Table 4.1). Similarly, male copulation attempts, which were observed in 8 trials, occurred more often with control females (control females = 6 and with pterin-extracted females = 2), although this small sample size did not indicate a significant effect of female treatment ($\chi^2 = 2.00$, $df = 1$, $P = 0.16$).

Male courtship behavior was affected by the male's nutritional status. First, low nutrition males seemed more eager to mate, performing more mating approaches (toward the same female pair) compared to high nutrition males (Fig.

4.4). Second, low nutrition males approached the control females significantly more often than did high nutrition males (Fig. 4.3 and Table 1; odds ratio = 0.497).

Discussion

Although male mate choice has recently been acknowledged as a potentially important form of sexual selection, numerous questions about its evolutionary causes and consequences remain unanswered (Bonduriansky 2001, 2009; Edward and Chapman 2011). For example, the effects of male mating preferences on the evolution of female ornaments remain poorly understood. In this study, we examined male choice in gift-giving *P. rapae* butterflies by combining an experimental manipulation of female wing coloration with alteration of male nutritional status. Our results demonstrate that males preferentially approached and attempted to copulate with females that had more colorful wings (higher pterin content). Further, this preference was stronger in males that had been subjected to short periods of starvation as larvae. Finally, a study of field-collected females revealed that wing coloration (but not body size) was positively correlated with female egg load, which supports the hypothesis that this ornament has evolved through direct selection via male choice.

Results of this study provide solid empirical evidence for exaggeration of pterin-based wing coloration in *P. rapae* females being at least partially driven by male mate choice. Male mating preferences in insects typically have been associated with traits directly linked to female fecundity, such as body and abdomen size (Bonduriansky 2001), but few studies have identified the specific

traits targeted by male choice. For example, LeBas et al. (2003) found that in the empidid dance fly *Rhamphomyia tarsata*, males select females based on the size of their pinnate leg scales, a trait that correlates with female fecundity. In butterflies, previous work using female models (dummies) made of paper or detached female wings have suggested that male mating preferences could be based on different aspects of female wing color (Silberglied and Taylor 1978; Rutowski 1980, 1981; Knuttel and Fiedler 2001; Ellers and Boggs 2003; Obara et al. 2008). Color-based preferences of both sexes were comprehensively investigated in the Checkered White butterfly, *Pieris protodice*, by Rutowski (1981) using dummies constructed from pterin-extracted and control wings. Free-flying males exhibited shorter chase durations when presented with models sporting pterin-extracted female wings. These studies suggest that male mate choice based on female wing coloration may be widespread in butterflies. However, as noted by Silberglied (1984), the use of female dummies limits observation of male behaviors to early courtship stages and male courtship approaches can be easily confounded with other behaviors (e.g. aggression, roosting) . By using live females in a paired-choice design, we were able to clearly identify male mating preferences during different stages of courtship (e.g. mating approaches and copulation attempts) and thus provide conclusive evidence that male butterflies use female wing coloration as the basis for mate choice.

Our results also indicate that preference for female ornaments in *P. rapae* depends on a male's larval nutrition. We found that low-nutrition males were more active (they approached females more often) and also had a stronger preference for

colorful females (approached colorful females more often) than high-nutrition males. This is a surprising result since individuals in poor condition are expected to reduce their sampling effort or choosiness (e.g. Hardling and Kokko 2005; Real 1991). One possible explanation for heightened preference shown by low-nutrition males is that such males may focus their mating effort toward early reproduction (e.g. first mating). Because *P. rapae* males deprived of food during the larval stage have shorter adult lifespans and therefore may have fewer mating opportunities (Tigreros 2013), early mating effort (including sampling effort) increases their chances of reproducing before they die. This result also suggests that the fitness benefits obtained from mating with more colorful females may be greater than the costs males may incur from sampling and choosing mates. Understanding variation in male mate preferences can have major impact on the evolution of female traits (Cotton et al. 2006).

The extent to which female ornaments signal benefits to males is poorly understood (Bonduriansky 2001; Nordeide et al. 2013). Theory suggests that ornament evolution in females should be more complex than in males (Chenoweth et al. 2006). To ensure honest signaling, female ornaments are expected to be costly, yet not to the extent that fecundity is reduced (Fitzpatrick et al. 1995; Kokko 1998, Chenoweth et al. 2006). In a recent review of female ornaments in vertebrates (mainly birds), Nordeide et al. (2013) found that carotenoid-based ornaments were often negatively correlated with female fitness benefits (offspring quantity or quality), while non-carotenoid-based signals were more often positively associated with female fitness. These authors propose that in the former

case, females face a tradeoff between allocating limited carotenoids (obtained through diet) to either ornaments, where they improve mating success, or to eggs, where they may improve offspring survival. Herbivorous insects, like butterflies, can only acquire limited amounts of nitrogen from their diets (Mattson 1980, Scriber & Slansky 1981; Slansky & Rodriguez 1987; Awmack & Leather 2002). Nitrogen is essential both for the production of pterins-based ornaments (Kayser 1985) and for vitellogenesis (Rivero & Casas, 1999; Shapiro & Ferkovich, 2002; Wall et al. 2002). Previous studies have shown that when *P. rapae* females are reared on low nitrogen diets, they significantly decrease nitrogen allocation to wings (Morehouse 2009; Tigreros et al. 2013) and abdomen size (a proxy for female fecundity) (Tigreros et al. 2013). Thus, similarly to carotenoid-based ornaments in many birds and fish, allocation to pterin-based ornaments could be expected to reduce female allocation to egg production in *P. rapae*. However, contrary to what would be expected based on Nordeide et al. (2013), in this study we detected a positive association between wing coloration and female fecundity. We propose two non-exclusive reasons for the lack of a tradeoff between female ornaments and fecundity in *P. rapae*. First, females may be able to compensate for nitrogen allocated to ornaments by acquiring nitrogen-rich nuptial gifts when mating. In these butterflies, nuptial gifts are assimilated by females, providing an extra source of nitrogen for fecundity and somatic maintenance. Second, maintenance of female fecundity may select for evolution of ornaments that are just moderately exaggerated. Although in *P. rapae* both sexes produce pterin-based wing ornaments, female ornaments are less exaggerated and require about

~50% less of the adult nitrogen budget compared to male ornaments (Morehouse 2009). Pterin pigments are abundant in nature; they are responsible for color ornaments in many butterflies (e.g. Rutowski et al. 2005; Wijnen et al. 2007), as well as reptiles (Steffen and McGraw 2007; Weiss et al. 2007), amphibians (Obika and Mbagnara 1964), and possibly birds (Thomas et al. 2013). Thus, while our results suggest selection on male mate choice for an honest pterin-based female ornament in *P. rapae* butterflies, the potential for honest pterin-based signaling still remains to be explored in most other taxa (but see Weiss et al. 2009, 2011).

Although female ornaments should be likely to evolve in nuptial gift-giving species (Bonduriansky 2001), few studies have provided empirical evidence for the occurrence of female honest signal in these species. This study illustrates the occurrence of a nitrogen-based female ornament in *P. rapae*, a species whose mating system includes nitrogen-rich nuptial gifts. Studies examining the link between the resources allocated to ornaments and those obtained through the male nutrient donations should further our understanding on the evolution of male mate choice and female ornamentation.

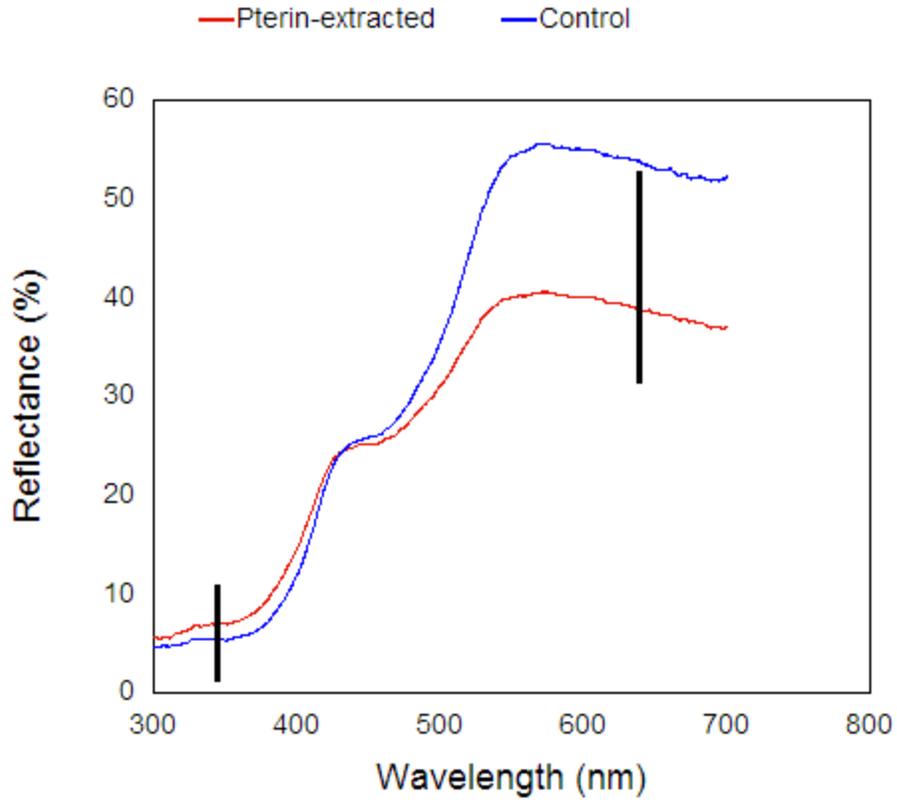


Figure 4.1. Average spectra of a representative sample of pterin-extracted (n=4) and control (n=4) *P. rapae* females. Vertical black lines indicate the range of natural variation observed for the UV and the visual range measured on field-collected females (n=43).

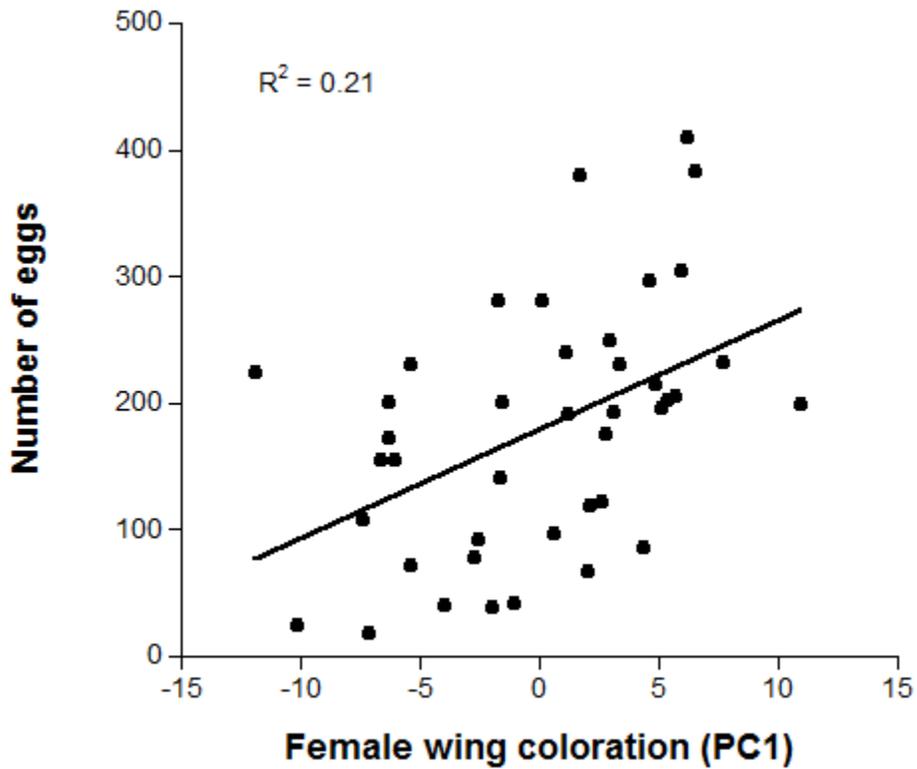


Figure 4.2. Relationship in field-collected *P. rapae* females between egg load (total number of eggs carried) and pterin-based wing coloration represented by first principal component explaining 92% of color variation (Factor loadings: visual reflectance = 0.99, UV reflectance = -0.13).

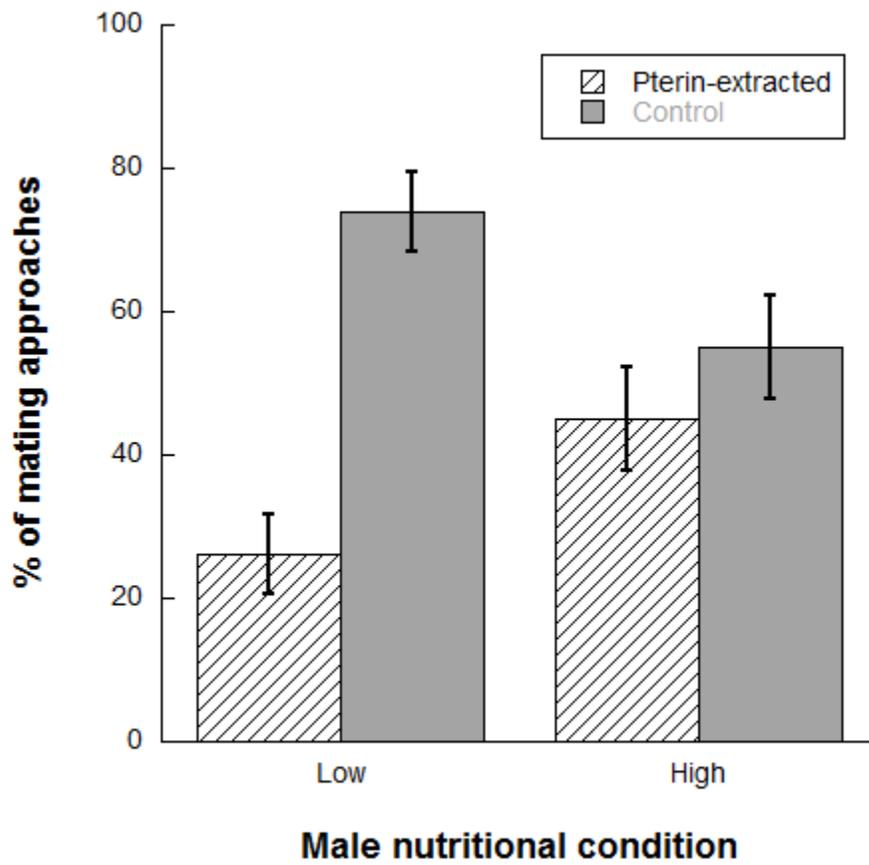


Figure 4.3. Percent of mating approaches (out of 168) of high-nutrition (n=16) and low-nutrition (n=16) *P. rapae* males toward females with either pterin-extracted or intact wings.

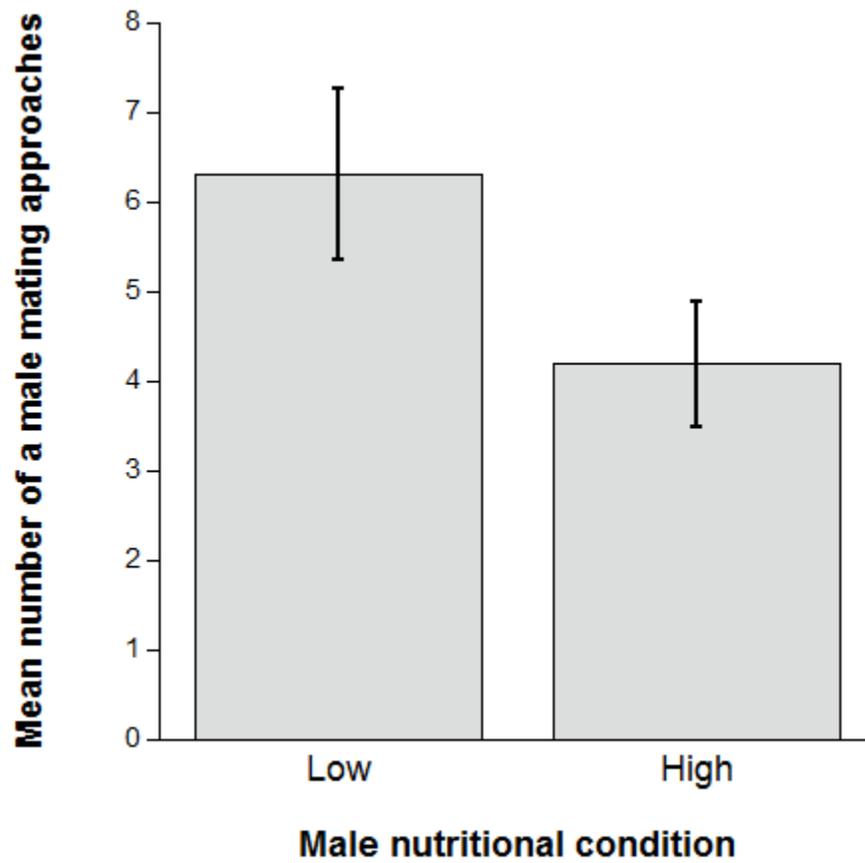


Figure 4.4. Bar graphs indicate mean (\pm SE) number of approaches by low-nutrition (N= 16) and high-nutrition (N=17) *P. rapae* males towards a pair of females.

Table 4.1. Logistic regression models showing the overall probability of *P. rapae* males directing mating approaches towards the control female (each male was given the choice between a sized-matched control and pterin-extracted female), and effect of male nutrition on the probability of approaching control females.

		Estimate	se	df	P
Probability of approaching control females	Intercept	0.61	0.16	1	0.0001
Effect of male nutrition on probability of approaching control females	Male nutrition	-0.35	0.16	1	0.03
	Intercept	0.56	0.16	1	0.0007

Chapter 5: Modelling sex-specific strategies for reproductive allocation: mate acquisition vs. nuptial gift/ offspring investment

Introduction

Resources in nature are often limited, so organisms are constrained to invest in certain traits at the expense of others, leading to resource allocation tradeoffs (Van Noordwijk and Dejong 1986). For sexually reproducing organisms, the primary decision about resource allocation concerns how much of the available resources will be spent on reproduction instead of on somatic tissue and maintenance (e.g. growth, survival). Evidence for such allocation tradeoffs among various life-history traits is well-established (e.g. Stearns 1992; Roff & Fairbairn (2007).

Yet tradeoffs may also be required within the pool of resources allocated to reproductive activity. Thus, there may be tradeoffs between traits associated with mate acquisition, such as ornaments or weaponry, vs. traits associated with offspring production, such as fecundity in females and producing gametes or nuptial gifts, or providing parental care (e.g. Moczek and Nijhout 2004). Indeed, a growing body of evidence suggests that males and females face tradeoffs between courtship signals that mediate mating success and ejaculate quality (e.g. Evans 2010; Klaus et al. 2011; South & Lewis 2012) and fecundity in females (e.g. Sappington, & Taylor 1990).

Although individuals of both sexes should allocate resources in ways that maximize their lifetime fitness (Collins 1980), based on their divergent

reproductive roles males and females should differ in their resource allocation to different components of reproductive success. According to classical evolutionary theory (Bateman 1948, Trivers 1972), males should maximize fitness by investing in traits that enhance their mating success, while investing little in nutritional gifts or paternal care. In contrast, females are expected to allocate their reproductive resources toward maximizing the number and quality of eggs and offspring they produce. However, empirical evidence from many taxa suggests that females can gain substantial direct benefits from multiple matings (South & Lewis 2011), and so might be expected to invest in traits that attract potential mates. Furthermore, male investment in ejaculate traits (quality and quantity), gifts, or paternal care can lead to enhanced fecundity (Arnold & Duvall 1994) and increased paternity share for males (reviews in South & Lewis 2012b).

Nuptial gifts constitute a type of male reproductive investment that is widespread across surprisingly diverse animal taxa (Boggs 1995, Vahed 1998, Gwynne 2008, Lewis & South 2012). Generally males produce or procure these gifts and transfer them to females during courtship or mating. In many species, nuptial gifts have been shown to increase female lifetime fecundity and/or longevity (reviews in South & Lewis 2012). These nutritive nuptial gifts thus provide a direct link between male and female resource budgets (Boggs 2009). Although gift production is costly for males and may limit their mating success, empirical studies have demonstrated several fitness benefits to males, including delayed female remating and increased paternity share when females mate multiply (Lewis & South 2012). Thus, because nuptial gifts act as a link between

male and female resource budgets, they have the potential to alter the allocation strategies used by both sexes.

Some empirical and theoretical work support the idea that allocation patterns in one sex might alter what allocation strategy is optimal for the opposite sex. A comparative phylogenetic study provided empirical evidence for correlated evolution between patterns of female allocation to flight vs. reproduction and male investment to nuptial gifts in fireflies (South et al. 2011). Across several lineages, whenever females lost their flight ability (presumably switching their resource allocation entirely toward reproduction), males subsequently lost their ability to produce nuptial gifts. Thus, in fireflies selection for male gifts appears to depend on patterns of female reproductive allocation. Similarly, a cross-taxonomic comparison in butterflies suggested that female allocation to reproductive reserves (fecundity) was lower for those species where males heavily allocated to nuptial gifts reserves (Karlsson 1995). Similar predictions were made based on theoretical work by Alonzo & Pizzari (2010), who developed a model to investigate how males tradeoff their resource allocation between sperm vs. fecundity-enhancing nuptial gifts. Their results showed that when females start out with high fecundity, males gain maximum fitness by allocating less to nuptial gifts and more to sperm production. However, their model was limited in that females could reach a maximum of only two matings, and sperm competition between time periods was ignored. Their model also failed to incorporate an important empirically demonstrated effect that nuptial gift size alters the female's likelihood of remating.

Thus, very few theoretical studies have examined optimal allocation strategies between male traits affecting mate acquisition (e.g. attractive courtship signals or ornaments, weapons used in intrasexual competition, searching for mates) vs. nuptial gifts. Similarly, few studies have considered optimal allocation by females toward traits such as ornaments to increase mate (and thus gift) acquisition vs. producing eggs (baseline fecundity). Finally, to our knowledge no work has investigated how allocation patterns prevalent in one sex might influence the optimal allocation strategy in the other sex.

In this study we used an individual-based modeling (IBM) approach to examine how patterns of reproductive allocation to either mate acquisition or offspring in one sex might alter the optimal allocation strategy used by individuals of the opposite sex. We based the biological features of our model on butterflies, holometabolous insects where resources required for adult reproduction are mainly acquired through juvenile feeding. As they near metamorphosis, such insects face allocation decisions that, once made, are irreversible. We chose an IBM approach in order to track the mating history of individuals over time, necessary because this altered individuals' mating thresholds, and resulted in complex sperm competition dynamics. This modeling approach also allowed us to fix the allocation pattern of one sex while introducing variation in allocation strategies among individuals of the opposite sex.

Methods

Biology of the Lepidoptera

Butterflies, acquire most of the resources used to grow and reproduce, during the larval stage. Larval resources, which are often limited need to be allocated to different reproductive traits (e.g. ornaments, weapons, ovaries, testis, accessory glands etc) during the larval and pupal stage. Limitation of larval resources has been shown to affect male and female allocation to adult traits, including those involved in reproduction (e.g. Tigreros 2013). Limitation of larval resources can reduce allocation to both traits associated with mate acquisition (e.g wing coloration) and traits associated with offspring production (e.g. female allocation to baseline fecundity and male allocation to nuptial gifts) (e.g. Tigreros et al. 2013; Tigreros 2013; Morehouse 2009) (Fig. 2.1).

As adults, temperate butterflies can live from 7-12 days (Scott 1973). During this time males and females can both mate multiple times. Male fitness is a function of his mating success as well as his ability to produce nuptial gifts (Fig. 2.1). Male mating success is determined by his investment towards mate acquisition, usually based on male wing ornamentation (Rutowski and Moreshouse 2010) During each mating, male Lepidoptera produces a nuptial gift in the form of a package that contains nutrients and sperm (spermatophore). These nuptial gifts can represent between 1.5 and 24% of the body weight of virgin males (Sims 1979, Boggs 1981; Sviird and Wiklund, 1986, 1989; Oberhauser, 1988, 1992; Wiklund and Forsbert 1991) and be equivalent to the amount of nitrogen in approximately 50–80 eggs (Karlsson 1998; Wiklund et al. 1998; Wedell and Karlsson 2003). Spermatophores increase male fitness in two different ways. First, larger spermatophores increase a male paternity share by

increasing female refractory period to re-mate, which results in fertilizing a greater proportion of eggs, and by increasing his fertilization success (e.g. Bissoondath and Wiklund 1997). The last male to mate with a female usually fertilizes the majority of further eggs laid (Simmons 2001), and males with larger spermatophores fertilize the majority of eggs. Second, as nutrients are incorporated into eggs, spermatophores are a form of parental investment (Boggs and Gilbert, 1979; Boggs, 1981; Boggs and Watt, 1981; Greenfield, 1982).

Because nuptial gifts increase a female's lifetime fecundity, female fitness is determined not only by her own allocation to fecundity, but also by her mating success (allocation to mate acquisition (Fig. 1). Multiply mated females may exhibit higher fecundity, longevity and egg weights (Watanabe, 1988; Oberhauser, 1989; Wiklund *et al.*, 1993). Females can have ornaments and solicit mating (e.g. Rutowski 1980) in order to attract mates and thus acquire multiple spermatophores.

Model structure

We developed an individual-based, stochastic simulation model using MATLAB software. Parameters and variables were informed by our own and published empirical data on the biology of the Lepidoptera and are summarized in Table 1. The model is comprised of individuals characterized by the state variables : identity number, sex, resource allocation to fecundity, resource allocation to nuptial gifts, and resource allocation to mate acquisition. Males and females were tracked on a day-by-day basis, updating each individual status as required: mating

event, provisioning of nuptial gift, female offspring production, and male offspring siring.

Within each time step, five events are processed in the following order (Figure 2): 1) *Encounter of a potential mate*: 2) *Mating event* 3) *Male nuptial gift donation* 4) *Female oviposition* 5) *Paternity*. These events are described in detail below.

1. *Encounter of a potential mate*: males and females are paired in a random order, by drawing their IDs from a uniform distribution.
2. *Mating event*: When a male and a female meet, mating happened only if both sexes accept each other. We use min-threshold theory [14] to model the rule for mating decision. This means that a male or a female will accept to mate with the first individual of the opposite sex they encounter whose attractiveness (Y) is above his/her minimum acceptance threshold.

We assume that individuals can only assess a potential mate based on his/ her investment in mate acquisition but cannot assess other traits of the individual such as fecundity or mating history. For males, acceptance was a function of a fixed minimum acceptance threshold ($MinThr_{male}$), and female allocation to mate acquisition (Y_{female}).

$$P(\text{Accepting}_{female}) = \begin{cases} 1, & \text{if } Y_{female} \geq MinThr_{male} \\ m(Y_{female}), & \text{if } Y_{female} < MinThr_{male} \end{cases}$$

For females, acceptance was a function of a fixed maximum acceptance threshold, a minimum acceptance threshold (MinTrh), and male allocation to mate acquisition. Female minimum acceptance threshold changes as a function of whether she has mated before, and if so, how much nuptial gift she has left from previous matings. At time t , female minimum threshold is a function of all the nuptial gifts she has received in the past ($t-1$) and the time when those gifts were obtained (j). Because females use nuptial gifts resources to produce eggs, the size of the nuptial gifts decreased 20% at every time point.

$$MinThr_{female_t} = 1 - \sum_{j=1}^{t-1} \left[\frac{NG_j * 0.8^{t-1-j}}{G_{max}} \right]$$

Female mating acceptance then, can be calculated as

$$P(Accepting_{male}) = \begin{cases} 1, & \text{if } Y_{male} \geq MaxThr_{female} \\ m(Y_{male}) + b, & \text{if } MinThr_{female} < Y_{male} < MaxThr_{female} \\ 0, & \text{if } < MinThr_{female} \end{cases}$$

3. *Male nuptial gift donation*: Whenever a male mated, he donated a nuptial gift (NG_t) nuptial gift donated at time t , was equal to half of the male resources available to produce nuptial gifts (F_{male}) at time t .

4. *Female oviposition*: In every time period a female that had mated at least once, oviposited. However, virgin females (mating_t = 0) did not lay any eggs. The

number of eggs laid in time t (E_{female_t}) was function of the female instantaneous fecundity ($InstF$) and/or the nuptial gift size she receives in that time period.

$$E_{female_t} = \frac{InstF}{10} + NG_t$$

If female did not mate in time period t , but had previously mated then laid eggs only as a function of her own allocation to fecundity.

$$E_{female_t} = \frac{InstF}{10}$$

5. *Paternity*: After mating, each male sired a proportion of the female eggs ($E_{last-male}$). The number of eggs the male sired in a time period depended on number of eggs female laid at time t (E_{female_t}) and proportion of eggs fertilized (C_t). Proportion of eggs fertilized (C_t) is defined with a pattern of last male sperm: last male will always sire most or the same number of eggs than previous males; minimum C_t will be constraint to range between 0.5-1. Variation within this range depending on a positive linear relationship with relative gift size G_t / G_{max} :

$$C_t = 0.5 + 0.5 \frac{G_t}{G_{max}}$$

If the female is a virgin at time t , then the male will sire all her offspring ($C_t = 1$). However, if the female has mated previously, then the male will fertilize a proportion of the female offspring ($C_t < 1$), and this value depends on the size of the nuptial gift that the male is donating (NG_t). Larger nuptial gifts will confer a larger proportion of offspring. Thus the last male that mates with a female will always father a number of offspring ($E_{last-male}$) that is a function of proportion of eggs fertilized and female instantaneous fecundity.

$$E_{\text{last-male}} = C_t * E_{\text{female}_t}$$

If female has previously mated, every previous male (no-last male) also fathers a smaller proportion of offspring she produces and will be a function of the proportion of offspring the last male fathers, female number of offspring produced, and the number of other males she has mated in the past.

$$E_{\text{previous-male}} = [(1 - C_t) / \text{matings}_{t-1}] * (E_{\text{female}_t}).$$

Model scenarios and model variations

The described model was run to evaluate male's optimal allocation strategy under three different female allocation scenarios:

- 1) 75% allocation to fecundity, 25% allocation to mate acquisition
- 2) 50% allocation to fecundity, 50% allocation to mate acquisition
- 3) 25% allocation to fecundity, 75% allocation to mate acquisition

Similarly, female's optimal allocation strategy was evaluated under three male allocation scenarios:

- 1) 75% allocation to nuptial gifts, 25% allocation to mate acquisition
- 1) 50% allocation to nuptial gifts, 50% allocation to mate acquisition
- 1) 25% allocation to nuptial gifts, 75% allocation to mate acquisition

We also show results for two model variations based on altering the male minimum acceptance threshold. 1) “*promiscuous males*” model, where the male minimum acceptance threshold was fixed to a relatively low value (0.2), and 2) “*choosy males*” model, where the male minimum acceptance threshold was fixed to a relatively high value (0.5). With a low $MinThr_{\text{male}}$ males accept almost all

type of females while with a high $MinThr_{male}$ males are more likely to mate with females that allocate more to mate acquisition (choosy males).

Because of the stochastic nature of the model, we ran each scenario 100 times and averaged the data (and SD) to give the reported results.

Results

Results of IBM simulations show that both male and female fitness, measured as lifetime number of offspring, were affected by their own allocation strategy as well as the allocation scenario of the opposite sex. In addition, different model results were obtained when male minimum acceptance threshold was set at a low level (*promiscuous males*) vs. a high level (*choosy males*).

Optimal allocation strategy for females

Promiscuous males: As expected, female fitness increased linearly with increased female allocation to fecundity and it reached a maximum when allocating ~80% (when males allocated 75% and 50% to nuptial gifts) or 90% (when males allocated 25% to nuptial gifts) to fecundity (Fig. 5.3A). However, after that, female fitness abruptly dropped across all the male-allocation scenarios. Although these females were highly fecund, they were less likely to mate because their allocation to mate acquisition (e.g. attractive ornaments) was below the minimum acceptance thresholds set for males (Fig. 5.3B). Interestingly, however, the highest female fitness resulted when males allocated 50% to nuptial gifts, rather than when males allocated more (75%) to nuptial gifts (Fig. 5.3A). This reflects the high rates of multiple mating of females when males allocate 50% to nuptial

gifts (Fig. 5.3B), in contrast with the low mating rates of females in the scenario when males allocated 75% to nuptial gifts (Fig. 5.3B). Thus, females optimized their fitness when males allocated moderately to nuptial gifts and females acquired several gifts by mating with multiple males.

Female mating success remained constant across most female allocation strategies, dropping abruptly when female allocation to mate acquisition fell below 20% (Fig. 5.3B). As mentioned above, because this high allocation to fecundity (~80-90%) made, females were unlikely to attract a mate. Female mating success was also affected by how much males allocated to nuptial gifts. When males allocated 75% to nuptial gifts (Fig. 5.3B), females had a very low mating rate. This may be at least partially driven by the effect that large nuptial gifts have on increasing female refractory period to re-mate.

Choosy males: When male mating threshold was set to a high level, female fitness reached maxima with slightly lower allocation to fecundity (Fig. 5.3C), compared to the *promiscuous males models* (Fig. 5.3A). When males allocated 25% to nuptial gifts, female optimum allocation to fecundity was 70%. When males allocated more to nuptial gifts (50-75%), female maximum fitness occurred at 60% allocation to fecundity (Fig. 5.3C). Similarly, female mating optimum was reached with lower allocation to fecundity (~50%) (Fig. 5.3D).

Optimal allocation strategy for males

Promiscuous males: Male fitness was maximized when allocation to nuptial gifts was 50% (Fig. 5.4A). From this intermediate maximum, male fitness decreased

with decreased allocation to mate acquisition as well as with decreased allocation to nuptial gifts. This pattern seems to be mostly driven by male changes in mating success (Fig. 5.4B), since changes in male fitness and mating success changed along the gradient of male allocation strategies (Fig 4A,B). Decreased mating success was associated with allocation to mate acquisition (which is expected), but also with decreased allocation to nuptial gifts. Because male require a nuptial gift to mate, reducing allocation to nuptial gift production also constrains male mating success (Fig. 5.4B). Also, as expected and independently of a male mating success, male fitness was highest in the scenario where females allocated 75% to fecundity and lowest when female allocation to fecundity was 25% (Fig. 5.4A).

Choosy males: The impact of high male choosiness was especially apparent for males in the scenario where females allocated 75% to fecundity (Fig. 5.4C,D). Fitness optimum for males was achieved at very low levels of allocation to nuptial gifts (~30%) (Fig. 5.4C), compared with 50% in the *promiscuous males* model (Fig. 5.4A). Mating success of these males was affected in a similar way (Fig. 5.4D). When females allocated 75% to fecundity, optimal mating success was achieved with a minimum allocation to nuptial gifts (~30%). Interestingly, across all three female-allocation scenarios, when females allocated 75% to fecundity, male showed the highest fitness success but the lowest mating success (Fig. 5.4D).

Contributions to male paternity from last vs. previous mates

Promiscuous males: Our modeling approach allowed us to keep track for each male of the contributions to lifetime fitness from each mating. For each male, lifetime fitness was the sum of the offspring that he sired when he was the last male to mate with a female (fitness as a last male) plus the offspring he sired when females subsequently re-mated with other males (fitness as a past male). Across all three female-allocation scenarios, fitness benefits gained as a last male were 3-4 fold higher than those gained as a past male (Fig. 5.5). Also, the optimal allocation strategy differed for fitness gained as a past vs. a last male (Fig. 5.5A, B). Maximum fitness gained as a last male was achieved when males allocated ~60% to nuptial gifts (Fig 6A). On the other hand, maximum fitness gained as a past male was achieved at much lower allocation to nuptial gifts (~30%; Fig. 5.6B). Males that produce large nuptial gifts benefit from increasing female fecundity and decreasing the chances she will mate with other males. On the other hand, even males that produce small nuptial gifts will benefit when the female re-mates with other males.

Choosy males: Increasing the male minimum acceptance threshold impacted the proportion of fitness obtained as a last vs. a past male, particularly in the scenario where females allocated 75% to fecundity (Figs 5.5C, D). For these males, the maximum fitness as last males was reached with lower allocation to nuptial gifts (< 40%) compared to males from the other scenarios (Fig. 5.5C) and from the *promiscuous males* model (Fig. 5.5A). Males under the 75% female-allocation scenario increased their fitness as last males by mating with highly fecund females that are very unlikely to re-mate due to their low allocation to mate

acquisition. Fitness obtained as a last male in other scenarios, where females are more attractive and more likely to mate (or for the *male promiscuous* model), is more dependent on male allocation to nuptial gifts, which impacts a female refractory period to re-mate.

Relationship between mating success and fitness

Promiscuous males (results for *choosy males* are similar, shown): The relationship between mating success and lifetime fitness was very different between males and females (Fig. 5.6).

Females achieved maximum fitness with two or three matings (Fig. 5.6A). However, the effect of extra matings on fitness, for the three male-allocation scenarios, remained constant for those females that mated more than three times. Females did not gain extra benefit from receiving more than three nuptial gifts, probably because those mating happened with males that had already used most of their nuptial gifts resources. When males allocated 75% to nuptial gifts than to mate acquisition, the majority of females mated only once and twice, and none had more than three matings (Fig. 5.6B). In contrast, when males allocated 50% to nuptial gifts, females mated up to seven times with most of the females mating four times (Fig. 5.6B). When males allocated less to nuptial gifts than to mate acquisition, females mated up to six times and mostly mated three and four times (Fig. 5.6B). However, fitness was either stable or decreased for those females that mated more than three times (Fig. 5.5A).

Across all three female allocation scenarios, males mated up to four times and their fitness increased with number of matings, the slope became steeper as female allocation to fecundity increased (Fig. 5.6C). Males show a relatively evenly distributed frequency of mating from zero to four times (Fig. 5.6D).

Discussion

In species whose mating system includes nuptial gifts, males and females can acquire benefits from investing in both mating success and offspring production (Lewis and South 2012). However, when resources are limited, tradeoffs between traits associated with mate acquisition and traits associated with offspring production and care may occur (e.g. South and Lewis 2012a). Because nuptial gifts act as a link between male and female resource budgets, they have the potential to alter the allocation strategies used by both sexes. This study examines how patterns of reproductive allocation to either mate acquisition or offspring production in one sex might alter the optimal allocation strategy used by individuals of the opposite sex.

Optimal allocation strategy for females

The evolution of attractive traits (e.g. ornaments) that favor a female's mating success has been considered unlikely due to the competing demands between these traits and female allocation to fecundity (Fitzpatrick et al. 1995; Chenoweth et al. 2007; Morales, Velando & Torres 2009; but see Doutrelant et al. 2008; Simmons & Emlen 2008). However, female ornamentation may evolve if it increases a female's chance of gaining material benefits in the form of male

nuptial gifts (Kokko and Johnstone 2002; Kraaijeveld et al. 2007). Major effects of nuptial gifts on female fitness have been reported in a variety of taxa. For example, in *Pieris napi* butterflies, females can double their lifetime fecundity when receiving large and/or multiple nuptial gifts (Karlsson 1998).

In this study, the importance of female allocation to mate acquisition was evident in the choosy males simulations. We found optimal allocation strategies that always included some sacrifice of resources from female fecundity towards mate acquisition. As predicted, female optimal allocation strategy was dependent on the allocation patterns used by males. When males allocated little to nuptial gifts (25%), females optimized their fitness with higher allocation to fecundity than when males allocated 50% or 75% to nuptial gifts (Fig 5.3C). Our results also suggest that females benefit the most by acquiring multiple, intermediate size nuptial gifts, rather than acquiring a single large nuptial gift.

Optimal allocation strategy for males

In butterflies, nuptial gifts can be seen as a form of parental investment as well as an adaptation that enhances a male's fertilization success relative to that of other males (Simmons 2001). In the majority of species females mate multiply and male ejaculates from various males compete for fertilizations (Parker 1970). The male benefit when allocating resources to nuptial gifts might be determined by the degree of female investment to fecundity, as well as her remating propensity.

We found that when females allocated 75% of their resources to fecundity males optimized fitness while allocating less to nuptial gifts (Fig. 5.4 C). Because these less-attractive female had a low propensity to remate (Fig. 5.3D), males

obtained most of their fitness benefits by being the last male to mate. This agrees with Parker's theoretical predictions that males would decrease allocation to ejaculates when under low risk of sperm competition (Parker et al. 1997), as well as previously suggested patterns of decreased allocation to nuptial gifts with female greater investment to baseline fecundity (Karlsson 1995; South et al 2010) and female monandry (Karlsson 1995). Alonzo and Pizzari's (2010) model also concludes that with small nuptial gifts, males increase their fitness if females do not remate. However, they did not take into account that nuptial gift size alters the female's likelihood of remating. Our model shows that in the scenarios where females were more likely to remate (Fig. 5.C: female allocation to fecundity 25% and 50%) male fitness was optimized by a moderated allocation to nuptial gifts.

Relationship between Mating success and fitness:

Bateman's (1948) classical experiment in *Drosophila melanogaster* suggested that male's fitness increases linearly with number of mating whereas female fitness levels off after a single mating. Using a modeling approach, Arnold and Duvall's extended Bateman's idea by examining how the relationship between mating success and fitness changed in the presence of nuptial gifts (Fig. 5.7). Their model showed that in mating systems with nuptial gifts, males as well as females, could benefit from extra matings.

However, previous models have failed to examine the relationship between male mating success and fitness under different female fecundity scenarios. In species where males invest heavily in nuptial gifts, empirical studies show that females are likely to show decreased investment in fecundity (Karlsson

1995; South et al. 2010). Our model demonstrates that although male fitness always increases with mating success, the rate of increase was smaller when females decreased allocation to fecundity (Fig 5).

Our study shows the complexities behind male and female allocation decisions and their interactions. This was particularly true for males, who seem to maximize their fitness in various ways. For example, when females allocate little to fecundity, males receive fewer benefits from the extra matings and are expected to increase allocation to nuptial gifts while decreasing allocation to mate acquisition. On the other side, because low allocation to fecundity also leads to female increased promiscuity, males should increase their allocation to nuptial gifts in order to compete for fertilizations with other males.

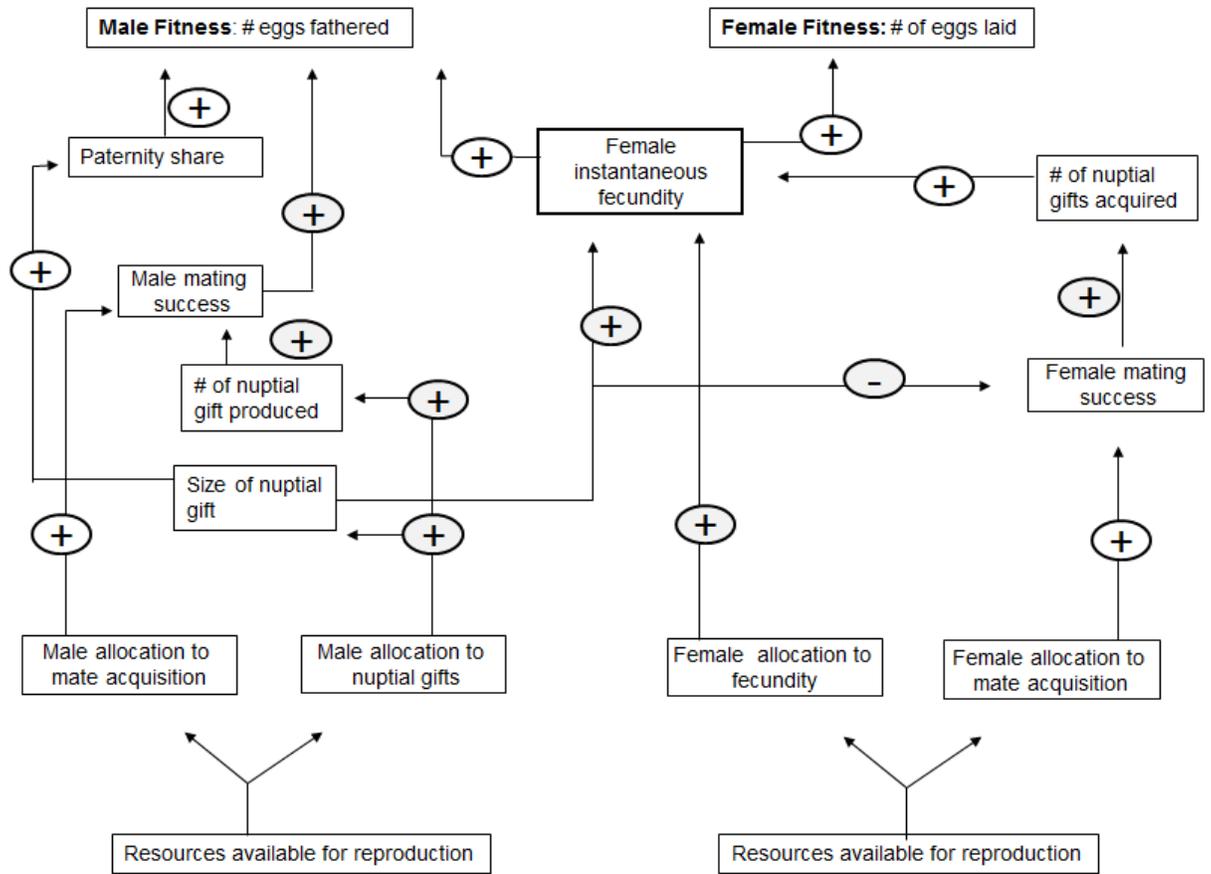


Figure 5.1. Signed digraph, based on the biology of the Lepidoptera, illustrates how allocation to different reproductive traits are associated with male and female mating success and fitness. + indicates a positive relationship while - indicates a negative relationship.

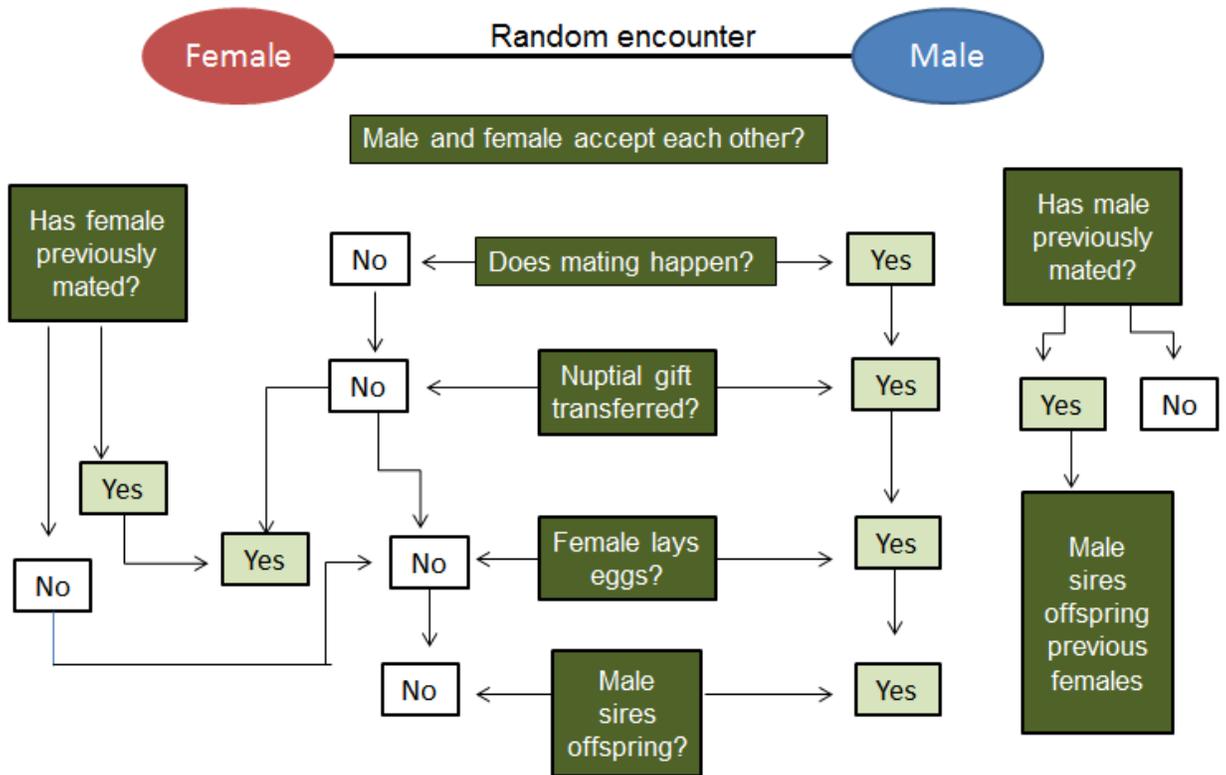


Figure 5.2. Simplified flow chart for the events that occur at each time step in each simulation model.

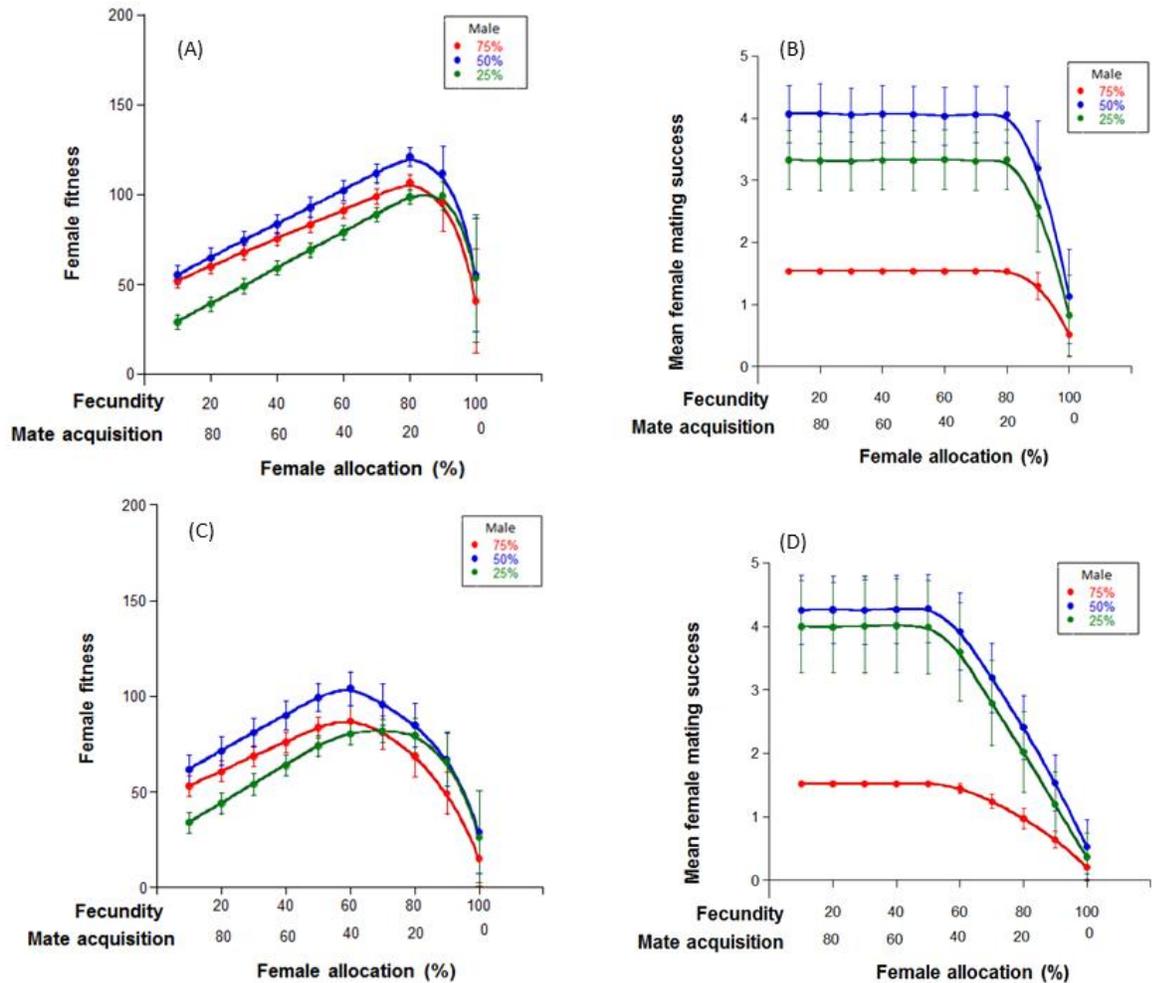


Figure 5.3. (A) and (C) Effects of female allocation to fecundity vs. mate acquisition on female fitness (measured as number of offspring produced) under three fixed male allocation scenarios: when allocation to nuptial gifts vs. mate acquisition is 75/25 (red), 50/50 (blue) and 25/75 (green). (B) and (D) Effects of female allocation to fecundity vs. mate acquisition on female mating success (measured as total number of matings) under three fixed male allocation scenarios: when allocation to nuptial gifts vs. mate acquisition is 75/25 (red), 50/50 (blue) and 25/75 (green). Figures (A) and (B) show the results from *promiscuous males* models ($MinThr_{male} = 0.2$) while figures (C) and (D) show the results from *choosy males* models ($MinThr_{male} = 0.5$).

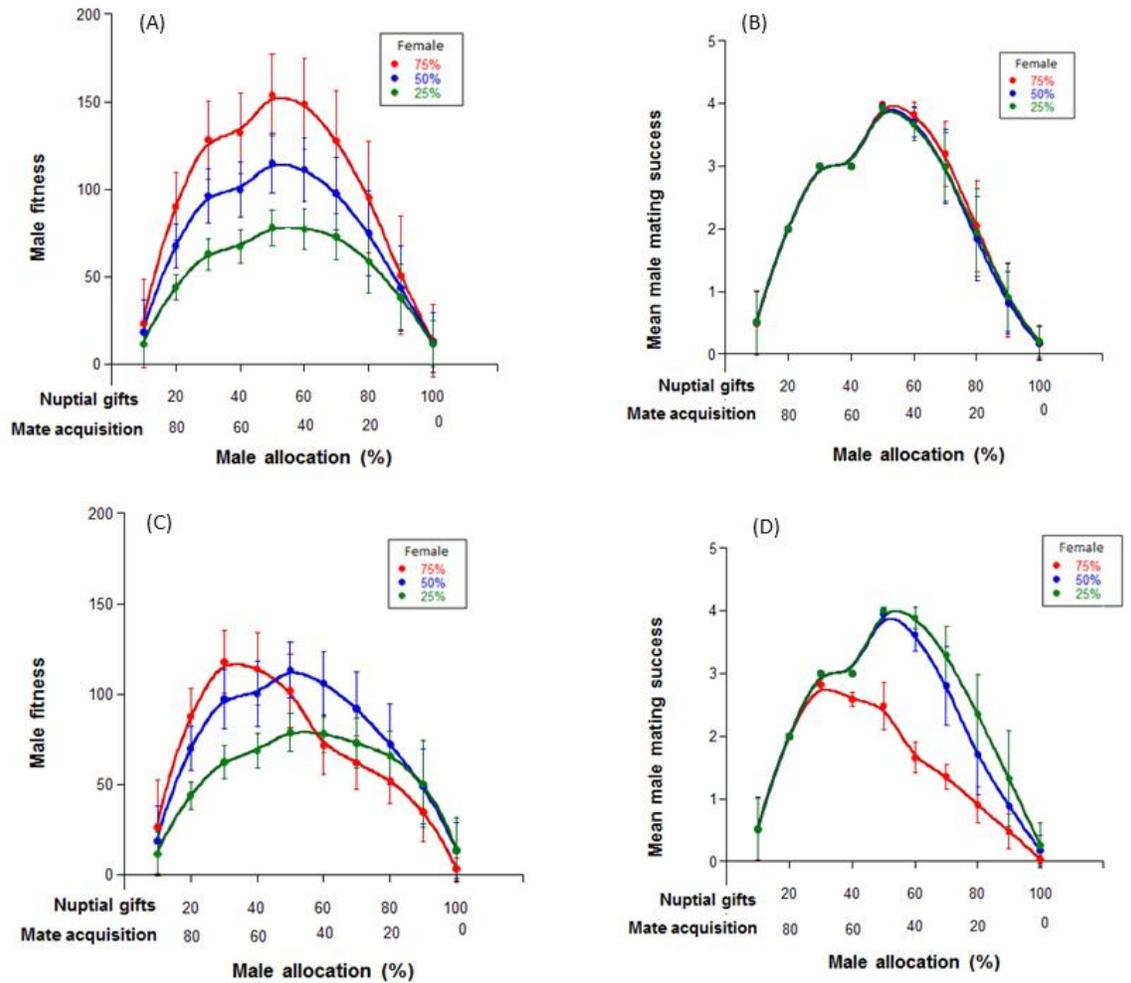


Figure 5.4. (A) and (C) Effects of male allocation to nuptial gifts vs. mate acquisition on male fitness (measured as number of offspring sired) under three fixed female allocation scenarios: when allocation to fecundity vs. mate acquisition is 75/25 (red), 50/50 (blue) and 25/75 (green). (B) and (D) Effects of male allocation to nuptial gifts vs. mate acquisition on male mating success (measured as total number of matings) under three fixed female allocation scenarios: when allocation to fecundity vs. mate acquisition is 75/25 (red), 50/50 (blue) and 25/75 (green). Figures (A) and (B) show the results from *promiscuous males* models ($MinThr_{male} = 0.2$) while figures (C) and (D) show the results from *choosy males* models ($MinThr_{male} = 0.5$)

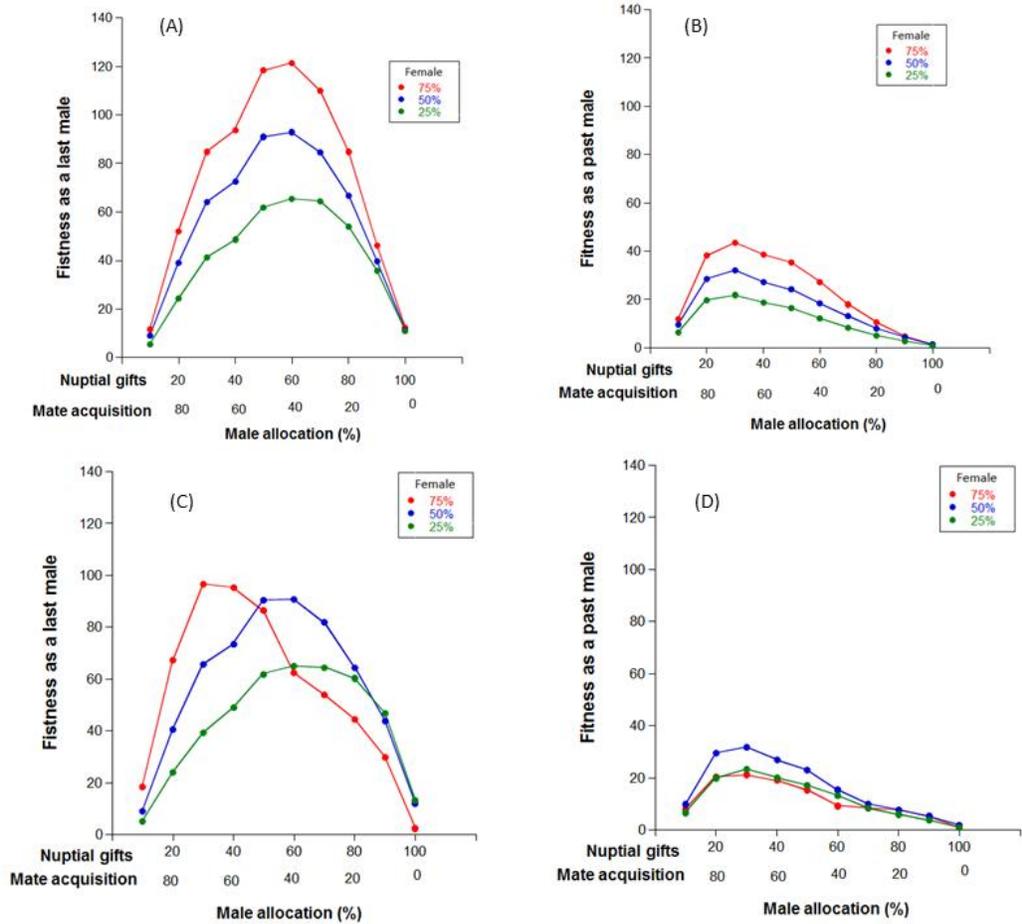


Figure 5.5. (A) and (C) Effects of male allocation to nuptial gifts vs. mate acquisition on fitness obtained as a last male (number of offspring sired when the male is the last male mated with a female) under three fixed female allocation scenarios: when allocation to fecundity vs. mate acquisition is 75/25 (red), 50/50 (blue) and 25/75 (green). (B) and (D) Effects of male allocation to nuptial gifts vs. mate acquisition on fitness as a past male (total number of offspring sired when the female a male has mated with re-mates with other males) under three fixed female allocation scenarios: when allocation to fecundity vs. mate acquisition is 75/25 (red), 50/50 (blue) and 25/75 (green). Figures (A) and (B) show the results from *promiscuous males* models ($MinThr_{male} = 0.2$) while figures (C) and (D) show the results from *choosy males* models ($MinThr_{male} = 0.5$)

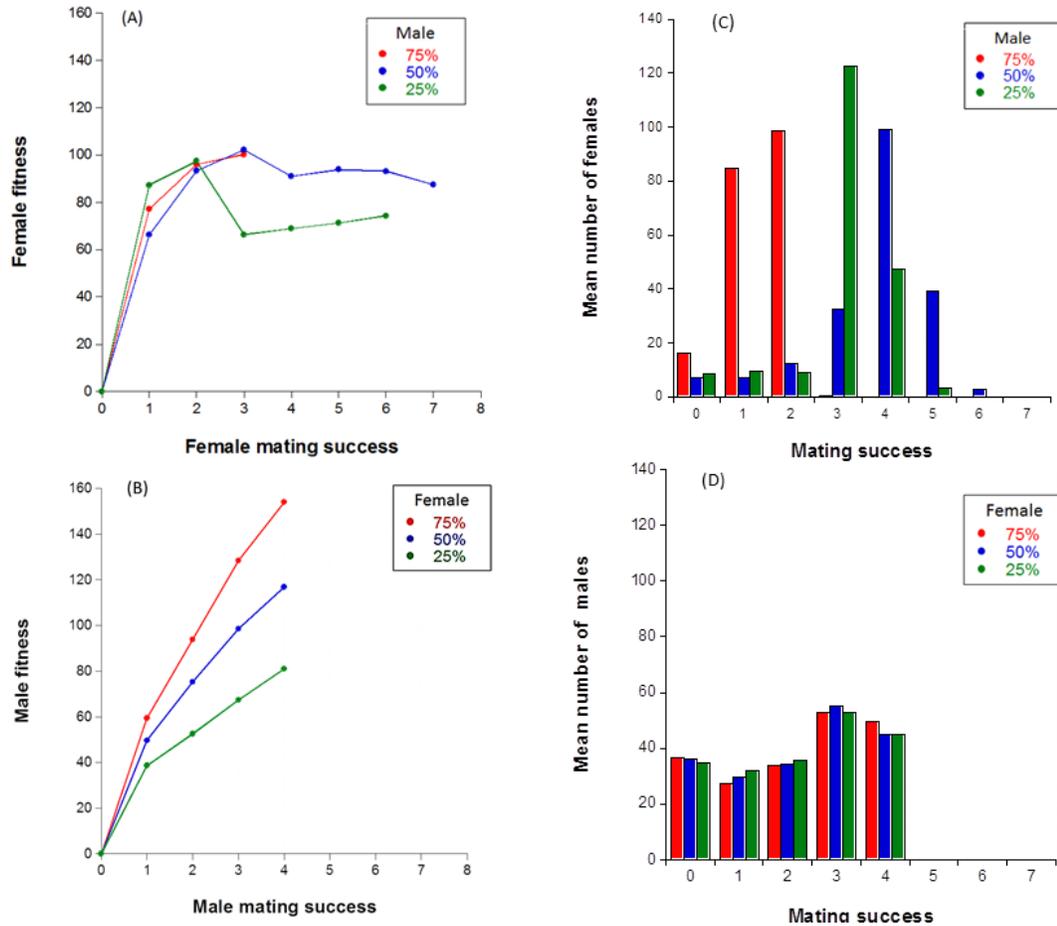


Figure 5.6 Effect of mating success on male and female fitness (A) Female fitness (No of offspring during lifespan) as a function of female mating success (Total number of matings)) under three different male-allocation patterns: when allocation to nuptial gifts vs. mate acquisition is 75:25% (red), 50:50% (blue) and 25:75% (green). (B) Male fitness (number of offspring fathered) as a function of male mating success (Total number of matings)) under three different female-allocation patterns: when allocation to fecundity vs. mate acquisition is 75:25% (red), 50:50% (blue) and 25:75% (green). (C) Frequency distribution of mating success among 200 females (D)Frequency distribution of mating success among 200 males.

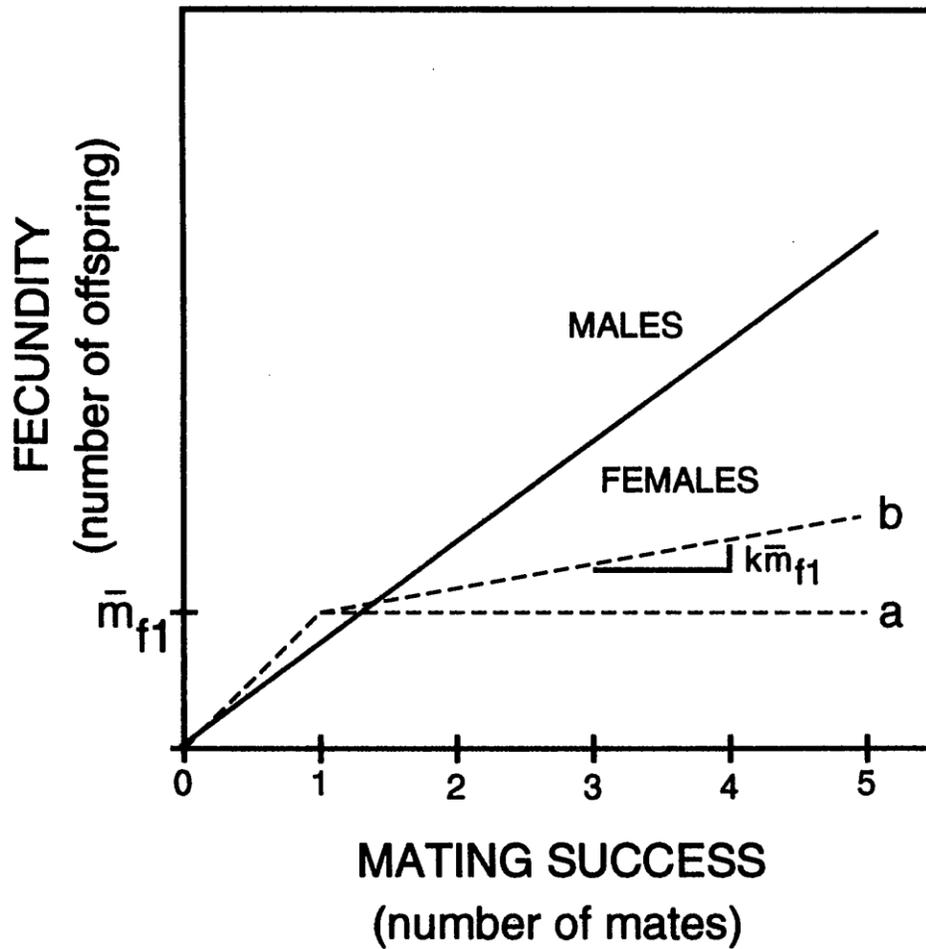


Figure 5.7. (Reprinted from Arnold and Duvall 1994) Relationships between fecundity and mating success in males and females when only females provide parental care. The relationship of fecundity to mating success is linear in males. The expected number of progeny produced by a female with one mate is \bar{m}_1 . When the male provides no nuptial gift, female fecundity does not increase if the female mates more than once (curve *a*). When the male provides a nuptial gift, female fecundity increases by the amount of $k\bar{m}_1$ with each mating beyond the first (curve *b*).

Table 5.1. Definitions for the parameters and variables in the models.

Parameters	Value	Definition
t	1-10	Ten discrete time periods
j	1-9	Time period when nuptial gift is acquired by female
R	100	Total resources available to reproduction
NGmax	100	Maximum potential size of a nuptial gift
MinThrmale	0.20 & 0.2	
MaxThrfemale	0.80	
MinFmale	5.00	Minimum available resources a male needs to mate
Variable	Value	Description
Y	0-1	Attractiveness
d	0-1	Proportion of resource allocated to Y
<i>Ffemale</i>	$1 - Y_{female}$	Maximum potential female fecundity
<i>Fmale</i>	$1 - Y_{male}$	Maximum potential male fecundity
<i>InstF</i>	$F_{female}/10$	a tenth of female allocation to fecundity
MinThrfemale	0-1	a function of female mating history

Chapter 6: Direct and correlated responses to artificial selection on sexual size dimorphism in the flour beetle, *Tribolium castaneum*

Abstract Sexual size dimorphism (SSD) is a conspicuous yet poorly understood pattern across many organisms. Although artificial selection is an important tool for studying the evolution of SSD, previous studies have applied selection to only a single sex or to both sexes in the same direction. In nature, however, SSD likely arises through sex-specific selection on body size. Here, we use *Tribolium castaneum* flour beetles to investigate the evolution of SSD by subjecting males and females to sexually antagonistic selection on body size (sexes selected in opposite directions). Additionally, we examined correlated responses to body size selection in larval growth rates and development time. After 7 generations, SSD remained unchanged in all selected lines; this observed lack of response to short-term selection may be due to evolutionary constraints arising from between-sex body size correlations. Developmental traits showed complex correlated responses under different selection treatments. These results suggest that sex-specific larval development patterns may facilitate the evolution of SSD.

Introduction

Sexual size dimorphism (SSD) is one of the most conspicuous and general patterns of phenotypic variation occurring in animal species (Fairbairn *et al.*, 2007; Stillwell *et al.*, 2010). SSD may evolve when both sexes are selected for different

expression of body size due to niche dimorphism, sexual selection, or divergent reproductive roles (Hedrick & Temeles, 1989; Fairbairn *et al.*, 2007). However, the evolution of SSD is complex because the response of body size to sex-specific selection might be constrained by between-sex genetic correlations (Lande, 1980, 1987; Chippindale *et al.*, 2001; Fairbairn *et al.*, 2007). Because males and females share autosomal genes, high genetic correlations of homologous traits between the sexes are commonly observed (Lande, 1980; Roff, 1997; Poissant *et al.*, 2010). As a consequence, selection in one sex can influence body size in the other sex, which would prevent each sex from attaining its optimal body size (Lande, 1980; Bedhomme & Chippindale, 2007).

Recently, there has been an increase in research aimed at elucidating the evolution of SSD and investigating its constraints. Some of these studies include comparative analysis (e.g. Meagher, 1992; Blanckenhorn *et al.*, 2007), quantitative genetics analysis (e.g. McDaniel, 2005; Rolff *et al.*, 2005), genetic manipulations (Chippindale *et al.*, 2001) and artificial selection experiments (e.g. Wilkinson, 1993; Reeve & Fairbairn, 1996; Delph *et al.*, 2004; Messina, 2004; Fox *et al.*, 2007). Artificial selection experiments are a powerful tool for studying SSD because contrary to other approaches that can only yield a snapshot of the evolutionary process by studying SSD on current populations, artificial selection has the potential to recreate how SSD might change over time. Previous studies using artificial selection to investigate the evolution of SSD (e.g. Reeve & Fairbairn, 1996; Delph *et al.*, 2004) looked for a change in SSD by either applying selection on body size in only one sex (e.g. Reeve & Fairbairn, 1996;

Daphne *et al.*, 2004), or by simultaneously selecting for larger body sizes in males and females (e.g. Alicchio & Palenzona, 1971; Reeve & Fairbairn, 1996). In these experiments, even though body size quickly responded to artificial selection, only a weak or no change in SSD was observed.

To date, artificial selection has not yet been used to explore a third important selective scenario for the evolution of SSD, which is selection acting antagonistically on body size between the sexes. In natural populations, selection frequently acts simultaneously but in opposite directions on body size in males and females (see Cox & Calsbeek, 2009). For example, in the house finch, *Carpodacus mexicanus*, where males are typically larger than females, Badyaev *et al.* (2000) found that fecundity selection acted to increase male body size but reduced female body size. In the water strider *Aquarius remigis*, where females are larger than males, Preziosi & Fairbairn (2000) and Fairbairn (2007a) found that fecundity selection favored larger females (with large abdomens), while selection favored smaller males (with small abdomen and thorax). By applying artificial selection simultaneously but in opposite directions to males and females, it might therefore be possible to disrupt the between-sex genetic correlations and alter SSD.

Additionally, adult body size is correlated with various developmental traits such as development time and growth rate (Stearns, 1992). In insects, an increase in body size is typically genetically correlated with increased development time and/or increased juvenile growth rate (Roff, 2000). Although various studies have shown that artificial selection on body size causes correlated

responses in developmental traits (e.g. Partridge & Fowler, 1993; Partridge *et al.*, 1999; Cortese *et al.*, 2002; Teuschl *et al.*, 2006), to our knowledge, no previous studies have looked for sex-specific responses in correlated traits.

In this experiment, we examined how SSD in the red flour beetle, *Tribolium castaneum*, changed when we applied short-term sexually antagonistic selection on body size to males and females. Like many insects (Stillwell *et al.* 2010), *Tribolium* females are larger than males and this sexual size dimorphism is evident in both pupal and adult stages (Sokoloff, 1977). We used pupal mass as a measure of body size, as this trait has been shown to have high heritability (Conner & Via, 1992; Via & Conner, 1995; Koncagül & Berger, 2006) and to be genetically correlated between the sexes (Via & Conner, 1995). We established three selection regimes in which we simultaneously selected male and female pupal mass in different directions: Increase size ($\uparrow F \uparrow M$), Increase SSD ($\uparrow F \downarrow M$) and Reverse SSD ($\downarrow F \uparrow M$). Based on previous work (Lande, 1980; Reeve & Fairbairn, 1996; Fairbairn, 1997b; Fairbairn *et al.*, 2007) we predicted that if SSD responded to artificial selection it would increase in both the Increase Size and Increase SSD treatments, while we expected SSD to decrease and eventually reverse direction in the Reverse SSD treatment. Also, we expected that selection on body size would produce correlated changes in development time and larval growth rate, and that such changes might differ between the sexes.

Materials and Methods

Beetles used in this experiment were derived from a synthetic strain we created to increase genetic variation available for selection. This synthetic strain was created by systematically crossing a laboratory strain with two strains recently collected from field populations (H-1 and P-1 strains provided by Dr. James Campbell, USDA). Throughout the experiment, beetles were maintained on enriched wheat flour (King Arthur; Norwich, Vermont) and kept in a dark incubator at 29°C, 70% relative humidity. We measured body size using 4-6 day pupal mass; during this time, pupal mass is relatively constant (unpublished data). Pupae were sexed under 20X magnification based on their genital lobe morphology. Pupae were weighed to the nearest 0.01mg (Mettler AT261 balance) and then kept individually in 0.5 ml microfuge tubes with flour until they became reproductive adults.

To examine how males and females respond to sexually antagonistic artificial selection on body size, we simultaneously selected both sexes for seven generations. We created four different selection treatments. We applied sexually antagonistic selection in two treatments: in one of these, we selected for increasing sexual size dimorphism (Increase SSD treatment; denoted $\uparrow F \downarrow M$), while in the second treatment we selected for reversed sexual size dimorphism (Reverse SSD treatment; $\downarrow F \uparrow M$). We also created a treatment to examine changes in SSD when both sexes were selected for higher pupal mass (Increase Size treatment; $\uparrow F \uparrow M$). As a control, a fourth treatment was created where parents for each generation were not selected on the basis of body size, but instead were randomly chosen. We maintained two replicate lines for each selection treatment,

for a total of eight lines. We used an individual selection design, measuring pupal mass for 80 males and 80 females within each line (a total of 1280 pupae per generation), and then choosing 15 males and 15 females with the most extreme phenotypes as specified by their selection treatment. This design yielded an estimated intensity of selection of 1.4, representing the mean deviation of selected individuals in phenotypic standard deviation units (Falconer and Mackay 1996).

Matings were set up between selected adults when they were 10 to 21 d post-eclosion. To avoid the possibility of assortative mating or sperm competition, 15 pairs were established by randomly assigning each male to mate with a randomly chosen female within each selected line. In the two control lines, individuals were chosen randomly to form 15 mating pairs. After a 48 h mating period, the 15 females were placed together in a jar with ~ 100 g of flour to lay eggs for 5 days. Females were then removed and larvae were allowed to develop with excess food until pupation.

In each generation, SSD was calculated as the difference between mean male and mean female pupal mass. We used control lines to adjust for changes unrelated to the selection regimes (e.g. environmental changes; data for control males and females is shown in online supplementary material). For each selected line, male and female pupal mass and SSD were corrected by calculating the difference between treatment and control means for each generation (Falconer & MacKay, 1996; Conner & Hartl, 2004).

After completing seven generations of artificial selection, we measured how male pupal mass, female pupal mass and sexual size dimorphism (SSD)

responded to selection. We analyzed the average response to selection by testing the slope of a regression line fitted to the generation means (testing the null hypothesis that the regression slope = 0; Falconer & MacKay, 1996). Slopes were calculated separately for each of the two replicates within each treatment, as well as for the two replicates pooled. We detected a slight increase in pupal mass of both sexes within the control lines (for males, regression slope $b = 0.03$ mg/generation, $p = 0.03$; for females, $b = 0.03$ mg/generation, $p = 0.02$). Such changes may have resulted from unexpected changes in the environment such as food quality or general management (Falconer & MacKay, 1996), and were taken into account in our corrected response to selection as explained above.

To look at phenotypic body size correlations, we calculated Pearson correlations between mean male and mean female pupal mass within the two replicate lines for each selection treatment for the seven generations.

These statistical analyses were conducted using SPSS software (SPSS Base 8.0 for Windows, SPSS Inc., Chicago).

Correlated responses

After seven generations of selection, we tested whether artificial selection on male and female pupal mass had produced correlated responses in developmental traits by measuring larval growth rate and development time for males and females. To measure correlated responses we used F_1 progeny of crosses between the two replicate lines of each selection treatment; combining replicates in this way reduces potential inbreeding effects (Partridge & Fowler, 1993). For each selection treatment, we randomly chose 10 males and 10 females

from each replicate, for a total of 20 pairs; we allowed them to mate, and collected approximately 4 eggs from each female (all eggs were laid within a 12-15 h period). These F₁ progeny were kept individually in vials with excess flour, and used to measure larval development time and growth rate. Development time was calculated as days from egg laying to pupation. Based on differences in larval growth rates that have been observed in *T. castaneum* (unpublished data) and other insects (Gotthard *et al.*, 1994) we separately estimated early and late larval growth rates. Using the *T. castaneum* average egg weight of 0.03 mg (Howe, 1968), along with individual larval mass at 14 d and pupal mass measured as described above, we calculated relative growth rates as follows:

$$\text{Early relative growth rate (early RGR)} = \frac{(\text{Ln } 14d \text{ mass}) - (\text{Ln } 0.03 \text{ mg})}{14 \text{ days}}$$

$$\text{Late relative growth rate (late RGR)} = \frac{(\text{Ln pupal mass}) - (\text{Ln } 14d \text{ mass})}{\# \text{ days}}$$

To test whether these developmental traits showed correlated responses to artificial selection on pupal mass, we used a mixed model approach (Proc Mixed in SAS 9.1.3; SAS Institute, Cary NC). For each of the three dependent variables (development time, early RGR or late RGR), we examined the overall effect of selection treatment and sex, both included as fixed factors. Because some individuals were from the same family (same mother and father), we also included a random term that accounted for this within-family correlation. We used pre-planned linear contrasts to compare the control with each of the 3 selection treatments (Control vs. Increase size, Increase SSD and Reverse SSD).

Results

Response to selection

Response to artificial selection on pupal mass of *T. castaneum* was rapid in both sexes. After seven generations of selection in the Increase Size treatment, mean pupal mass of males and females had increased by 70% (Fig.1A), with both sexes showing significant responses to selection (Table 1). However, in the two treatments where we applied sexually antagonistic selection, pupal mass did not show the expected response. In the Increase SSD treatment ($\uparrow F \downarrow M$), we observed no consistent change in pupal mass for either sex over seven generations (Fig. 6.1B); pooling the two replicate lines, females showed a slightly negative slope (Table 6.1). In contrast, in the Reverse SSD treatment ($\downarrow F \uparrow M$), both sexes showed a marked decrease in mean pupal mass (Fig. 6.1C); after seven generations of selection, males and females were 44% and 50% lighter, respectively, with both sexes in both replicates exhibiting a significant decline in response to selection (Table 6.1).

Sexual size dimorphism showed little change in all of the selection treatments (Table 6.1; regression of SSD across generations, all $P > 0.3$). Across seven selected generations, changes in average pupal mass of males closely matched those of females, and mean pupal mass of the two sexes showed significant positive correlations within each selection treatment (Fig. 6.2).

Correlated responses to selection

All three developmental traits showed significant differences among the selection treatments after seven generations (Early growth rate: $F_{3, 214} = 23.58$, $P < 0.0001$; Late growth rate: $F_{3, 212} = 6.21$, $P < 0.0005$; Development time: $F_{3, 215} = 20.55$, $P < 0.0001$). In the Increase Size treatment both sexes showed significantly higher early growth rates compared to controls after seven selected generations (Fig. 6.3A; $t = 7.31$, $p < 0.0001$). In this selection treatment no correlated responses were detected in either late growth rate (Fig. 6.3B; $t = -1.88$, $p = 0.06$) or development time (Fig. 6.3C; $t = 1.85$, $p = 0.07$). In contrast, in the Increase SSD treatment ($\uparrow F \downarrow M$), there was no change in early (Fig. 6.3A; $t = 0.53$, $p = 0.59$) or late (Fig. 6.3B; $t = -0.09$, $p = 0.92$) growth rates; however, there was a significant decrease in development time (Fig. 6.3C; $t = -2.09$, $p = 0.04$). In the reverse SSD treatment ($\downarrow F \uparrow M$), there was no change in early growth rate (Fig. 6.3A; $t = 1.57$, $p = 0.117$), although there was a significant increase in late growth rate (Fig. 3B; $t = 2.31$, $p = 0.02$), and a significant decrease in development time (Fig. 6.3C; $t = -5.38$, $p < 0.0001$). The faster late growth rate seems to be mostly driven by the females, although no sex-by-treatment interaction was detected (Fig. 3B; $F_{3, 212} = 1.21$, $P = 0.31$).

Discussion

This experimental study provides insight into SSD using a novel approach in which artificial selection on body size was simultaneously applied in opposite directions to males and females in *Tribolium* flour beetles. In nature, SSD is thought to evolve when sex-specific selection produces different body size optima

for males and females (Fairbairn *et al.*, 2007). However, when we attempted to alter SSD using short-term sexually antagonistic artificial selection, we found no change in either the Increase SSD ($\uparrow F \downarrow M$) or Reverse SSD ($\downarrow F \uparrow M$) treatments across seven selected generations. When both sexes were selected simultaneously for increased body size ($\uparrow F \uparrow M$), although beetles became larger we also found no change in SSD. Within-line mean male and female body size remained highly correlated in every selection treatment. Finally, we found that selection treatments produced correlated changes in different *Tribolium* developmental traits.

Previous artificial selection experiments studying SSD have applied directional body size selection to only a single sex (e.g. Reeve & Fairbairn, 1996; Delph *et al.*, 2004), or have simultaneously selected both sexes in the same direction (e.g. Reeve & Fairbairn, 1996). Artificial selection experiments looking at changes in SSD in *Drosophila* have found either no change or an unexpected decrease in SSD; for example, SSD decreased even though selection was applied to increase male size or increase size in both sexes (Zeleny 1921; Alicchio and Palenzona 1971; Partridge and Fowler 1993; Reeve and Fairbairn 1996; Teuschl *et al.* 2006). Delph *et al.* (2004) performed artificial selection in the dioecious plant, *Silene latifolia*, to determine how between-sex genetic correlations influence sexual dimorphism in flower size. In lines where male plants with larger flowers were selected for 4 generations, female flowers also became larger; similarly, when smaller female flower size was selected, males also had smaller flowers. This study documented high between-sex genetic correlations in flower size that appeared to limit any change in size dimorphism of *Silene latifolia*

flowers. In our study of *Tribolium beetles*, we also found no change in the degree of SSD even when we applied sexually antagonistic artificial selection on body size. Thus, although Reeve & Fairairn's (2001) genetic simulation model suggests that significant changes in SSD can occur over relatively short time scales, experimental results to date indicate that high degrees of sexual dimorphism are unlikely to evolve under short-term selection on body size.

Our *Tribolium* results showing constant SSD even in the face of sexually antagonistic selection support previous artificial selection experiments indicating that between-sex body size correlations constrain the evolution of high SSD. In the Reverse SSD ($\downarrow F \uparrow M$) treatment, both sexes exhibited reduced pupal mass; even though males were subjected to direct selection for increased size, their pupal mass declined as a correlated response to selection on females. In the Increase SSD ($\uparrow F \downarrow M$) treatment, neither sex responded to selection, suggesting that the effect of direct selection in one sex was counterbalanced by opposing selection in the other. Even with the sexually antagonistic selection that we applied in these treatments, the phenotypic correlation between male and female mean body sizes within each line remained high across seven generations. In a previous study (Via & Conner, 1995) moderately positive genetic correlations in body size were found between the sexes in *T. castaneum*, although measures varied between two strains and across five environments, with estimates ranging from $r_A = 0.08$ to 0.653. Although genetic correlations were not measured in our study, this evidence is consistent with the finding by Delph *et al.* (2004) that such correlations act to constrain the independent evolution of sex-specific body sizes.

Our results also provide insight into the physiological mechanisms generating the changes in body size we observed under different artificial selection regimes. We found several significant correlated changes in *Tribolium* beetles' developmental traits when we applied artificial selection on pupal mass. In the Increase Size treatment, larger body size for both sexes was associated with a significant increase in growth rates during early larval development (hatching to day 14) relative to controls; there was no change in development time or late growth rate. In the Reverse SSD treatment, smaller body sizes for both sexes were associated with significantly shorter development times but increased late growth rates. Similar correlated responses were found by Englert & Bell (1969), who observed that *Tribolium* beetles selected for large pupal mass showed increased early growth rates (represented as 13-day larval mass) while beetles selected for small pupal mass showed shorter development times. These results indicate that changes in *Tribolium* body size are mediated by distinct physiological mechanisms depending on whether size is increasing or decreasing. It also supports Nijhout's *et al.* (2010) mathematical model showing that the correlation between body size and development time is context-dependent and that as a consequence body size and development time can change independently of each other.

Body size is a complex polygenic trait and, even in the face of high between-sex correlations, the evolution of SSD may be facilitated by sex-biased expression of shared genes affecting the physiological mechanisms controlling growth and development (Badyaev, 2002; Mank, 2009; Stillwell & Davidowitz,

2010). Growth in holometabolous insects is restricted to juvenile stages, thus adult body size is determined by the size attained during the final larval stage. The physiological mechanisms that determine insect size at metamorphosis have been particularly well-studied in the tobacco hornworm, *Manduca sexta* (reviewed by Davidowitz *et al.*, 2005; Nijhout *et al.*, 2006; Shingleton *et al.*, 2007), where three factors have been shown to regulate body size: larval growth rate, a critical size threshold, and the duration of a terminal growth phase. Thus, changes in body size can arise through alterations during several stages in the growth process. Recent work has shown that sex differences in the genetic architecture and physiological mechanisms that control larval size contribute to the maintenance of SSD in *M. sexta* (Stillwell & Davidowitz 2010). Our results also provide support for the idea that SSD involves the evolution of sex-specific modifiers acting on shared developmental pathways (Badyaev, 2002; Stillwell & Davidowitz, 2010). In the reverse SSD treatment, females that were directly selected for reduced size showed 9% higher late growth rates compared to males from the same selected line, whose body sizes also decreased. Although this difference was not statistically significant, it does suggest the existence of a sex-specific compensatory mechanism to maintain body size in females.

In conclusion, artificial selection experiments provide a powerful tool that may contribute to understanding how selection acts on sex-specific patterns of growth and development. Additional work elucidating sex differences in growth and development will provide greater insight into how SSD is maintained and responds to selection.

Table 6.1. Linear regression slopes describing changes of cumulative response, over seven generations, of mean pupal mass for females, males, and SSD (calculated as $\bar{X}_f - \bar{X}_m$) for three different selection treatments: Increase male and female size ($\uparrow F \uparrow M$), increase sexual size dimorphism ($\uparrow F \downarrow M$), and reverse sexual size dimorphism ($\downarrow F \uparrow M$). Test of a H_0 of regression slope = 0. Regression test significant at * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Selection Treatments	Replicate	Response (slopes)		
		females	males	SSD
Increase Size ($\uparrow F \uparrow M$)	1	0.12***	0.115***	0.009
	2	0.075***	0.081***	-0.006
	Avg.	0.099***	0.098***	0.002
Increase SSD ($\uparrow F \downarrow M$)	1	-0.029**	-0.022*	-0.007
	2	-0.023	-0.023	-0.001
	Avg.	-0.026*	-0.022	-0.004
Reverse SSD ($\downarrow F \uparrow M$)	1	-0.064**	-0.061***	-0.003
	2	-0.051**	-0.049**	-0.002
	Avg.	-0.057**	-0.055***	-0.002

Figure Legends

Figure 6.1. Cumulative responses to artificial selection on male (dashed lines) and female pupal mass (solid lines) in the flour beetle *Tribolium castaneum* relative to control (unselected) treatment. Four selection regimes were used: A) Increase Size of both sexes ($\uparrow F \uparrow M$). B) Increase sexual size dimorphism ($\uparrow F \downarrow M$), and C) Reverse sexual size dimorphism ($\downarrow F \uparrow M$). Responses shown are calculated as the deviation of each selected line from the control means (unselected individuals). There were two replicates for each selection treatment (represented by open and closed symbols).

Figure 6.2. Relationship between mean male and mean female pupal mass (means were corrected by calculating their deviation from the controls) during seven generations. Pearson correlations were calculated for Increase Size ($r = 0.97$, $P < 0.0001$, $N = 14$), Increase SSD ($r = 0.91$, $P < 0.0001$, $N = 14$) and Reverse SSD ($r = 0.90$, $P < 0.0001$, $N = 14$) treatments.

Figure 6.3 Correlated responses of developmental traits when *T. castaneum* pupae were selected for Increase Size ($\uparrow F \uparrow M$), Increase SSD ($\uparrow F \downarrow M$), and Reverse SSD ($\downarrow F \uparrow M$). Panels show A) early relative growth rate (RGR), B) late RGR, and C) development time. Asterisks indicate treatments that differ significantly (following Bonferroni corrections) from the unselected control treatment.

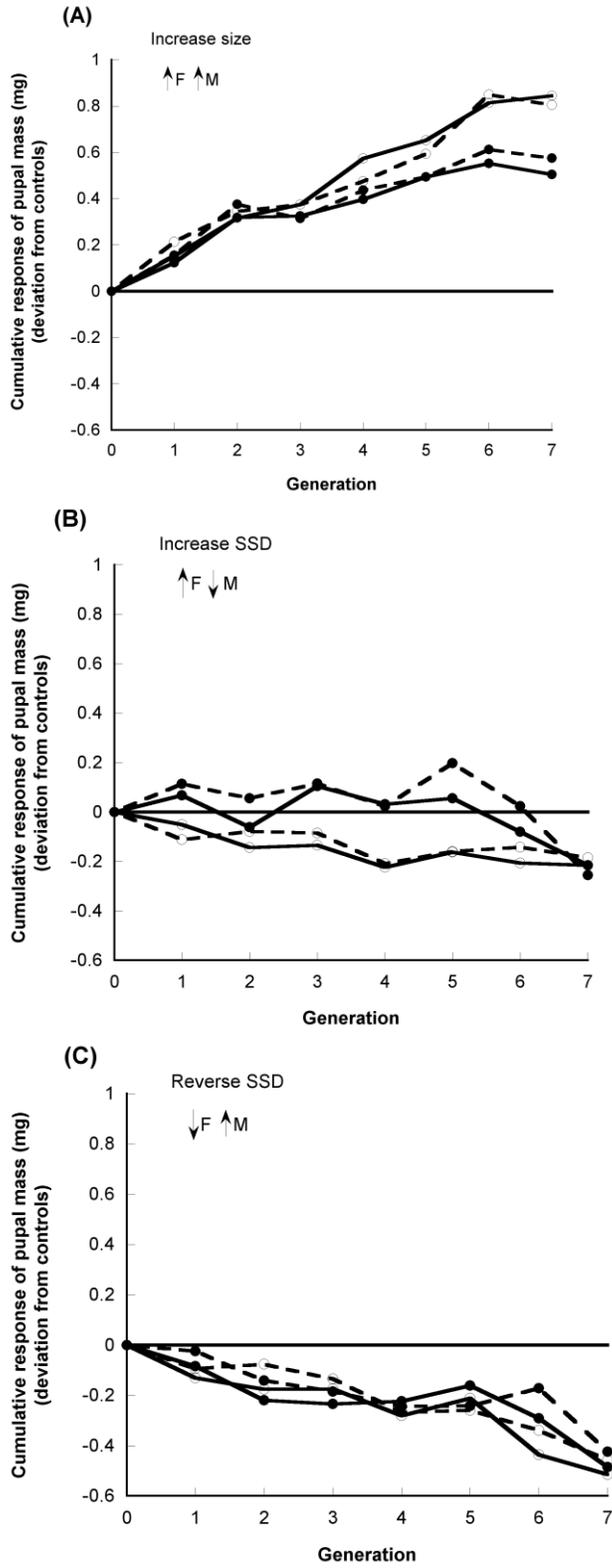


Figure 6.1

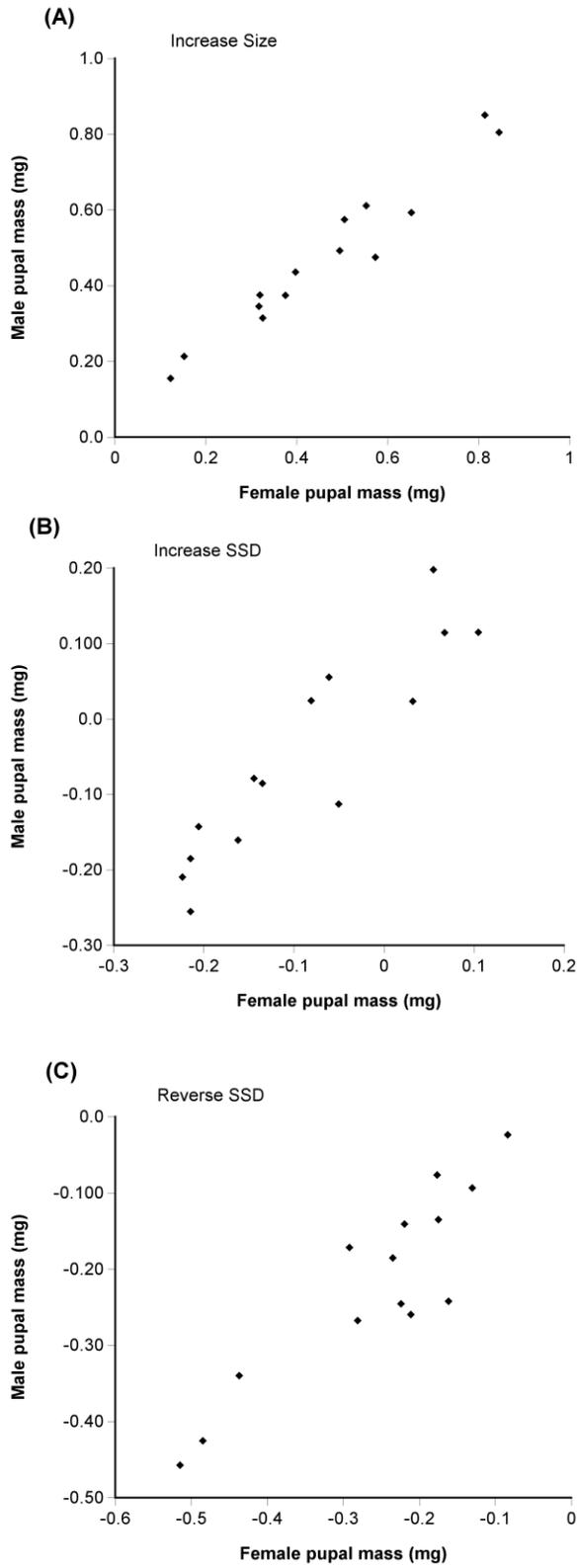


Figure 6.2

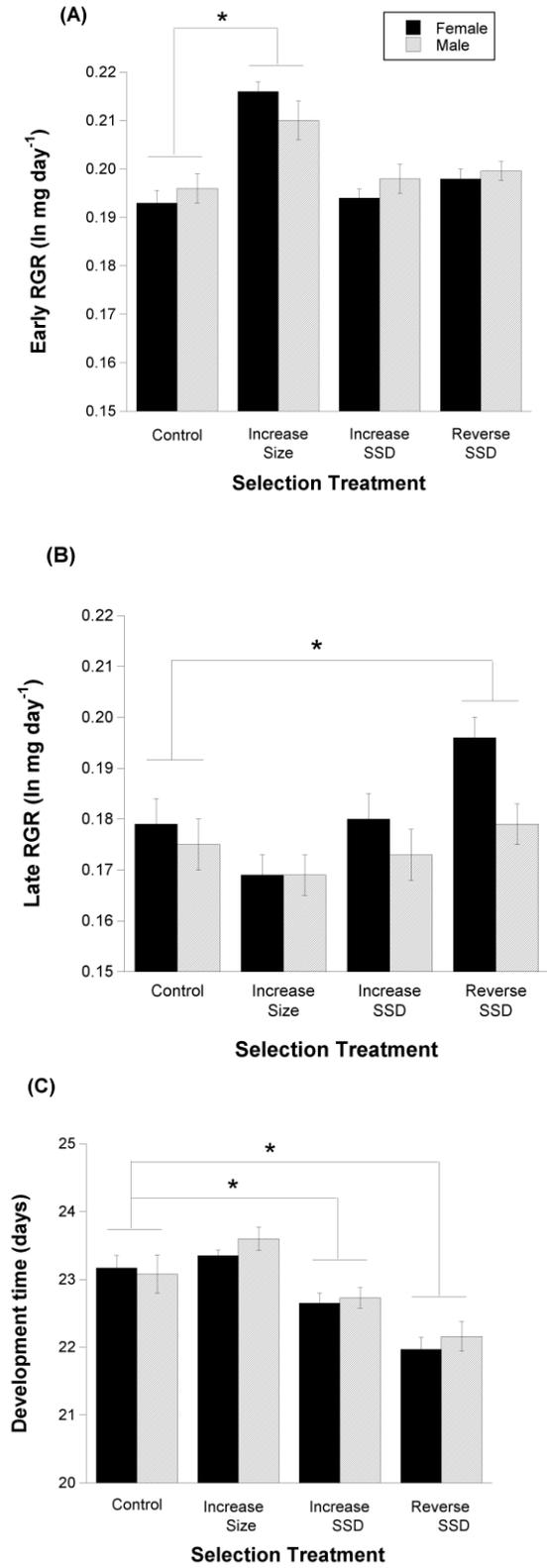


Figure 6.3

Chapter 7: Summary and Conclusions

The main goal of this dissertation was to reach an understanding of how non-traditional male and female sexual traits and behaviors can arise. Throughout each chapter I emphasized how nutrition and life history traits impact male and female sexual traits in insects with a complex life cycle. Results and significance of these findings are discussed below.

Evolutionary theory predicts that organisms will allocate resources in a way that maximizes their reproductive fitness (Stearns 1992; Roff 2002). In chapters 2 and 3, I performed diet manipulations to gain knowledge about the relative importance of primary and secondary sexual traits on male and female fitness. More specifically, I studied how variation in dietary nitrogen, a limited nutrient for herbivores, impacted different aspects of *Pieris* butterflies' fitness. I found that larval nutrition influenced adult fitness through three different pathways: altering time to metamorphosis, altering pupal size (size at transition) and latent effects carried over into the adult. Interestingly, the fitness impact of limited nitrogen larval diet was neither entirely positive nor negative. For example, although low-nitrogen males sacrificed wing coloration (trait that increases male mating success via female choice), low-nitrogen males allocated more protein to their spermatophores (increasing parental investment).

Responses to early food constraints differed between males and females (Ch 3). Limitation of dietary nitrogen during the larval stage decreased female but not male allocation to primary reproductive traits (e.g. abdomen). Even though allocation to fecundity is expected to directly affect female fitness, females may

be able to afford sacrificing resources to fecundity because they can compensate by acquiring male derived nutrients from nuptial gifts (Leimar et al. 1994). However, my results (Ch 3 and Ch 4) showed that male mating preferences for high quality females, limited female mating success (Ch 3). Therefore, females reared on low nitrogen diets were unlikely to acquire nuptial gifts.

The occurrence of male mate choice in this gift-giving butterfly (Ch 4) was an expected result, as male mating preferences are predicted to evolve when male mating investment is large (e.g. donation of nuptial gifts) (Bonduriansky 2001). More surprising, however, was the finding that male mate choice was driving exaggeration of female coloration and that this ornament honestly signaled female fecundity in *P. rapae* (Ch 4). Because investment in sexually-selected signals has the potential to carry high fecundity costs, evolution of female ornamentation has been thought unlikely to occur (e.g. Chenoweth et al. 2007; Morales, Velando & Torres 2009). However, evolution of female ornamentation might be possible in *P. rapae*, and similar species, if females are able to compensate for resources allocated to ornaments by acquiring those same resources from nuptial gifts when mating.

Male gifts can determine the magnitude of benefits females obtain from allocating resources to ornaments at the expense of fecundity. I further explored this idea using an individual based simulation based on lepidopteran biology. These results indicated that females benefit most by splitting allocation or resources to reproduction and ornaments (which allow them to acquiring several gifts). Additionally, by taking into account sperm competition, this model also

indicated that the benefit males obtain from nuptial gifts was determined by both the degree of female investment into fecundity, as well as her remating propensity.

Finally, in Ch 6, I used a different approach to determine the extent to which between-sex genetic correlations might constrain the independent evolution of traits in each sex. To do this I used *Tribolium castaneum* flour beetles and applied sexually antagonistic selection on body size, which is key for both male and female reproduction. My results supported the idea that between-sex genetic correlations constrain the independent evolution of body size. In contrast with results from previous chapters, this study suggests that expression of sexual behaviors and traits in one sex, can result from non-adaptive correlated response to selection on the other sex.

In summary, I have shown that the nutritional ecology of the sexes reflects and shapes male and female sexual roles. This work also revealed that non-traditional male and female sexual traits and behaviors are tightly linked to male investment and female acquisition of nuptial gifts. By testing various adaptive and non-adaptive hypotheses, I provide new insights into how non-typical male and female sexual traits and behaviors evolve.

Bibliography

- Alicchio R, Palenzona DL (1971) Changes of sexual dimorphism values in *Drosophila melanogaster*. *Boll. Zool.* **38**: 75-84.
- Alonzo SH, Pizzari T (2010) Male fecundity stimulation: conflict and cooperation within and between the sexes. *Am. Nat.* **175**, 174–185.
- Amundsen T, Forsgren E (2001) Male mate choice selects for female coloration in a fish. *PNAS.* **6**, 13155–13160.
- Amundsen T (2000) Why are female birds ornamented? *Trends Ecol. Evol.* **15**, 149–155.
- Andersson M (1986) Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* **40**, 804-816
- Andersson M (1994) Sexual selection. Princeton Univ. Press, Princeton, NJ.
- Apple JL, Wink M, Wills SE, Bishop JG (2009) Successional change in phosphorus stoichiometry explains the inverse relationship between herbivory and Lupin density on Mount St. Helens. *PloS ONE*, **4**, e7807.
- Arikawa K, Wakakuwa M, Qiu X, Kurasawa M, Stavenga DG (2005) Sexual dimorphism of short-wavelength photoreceptors in the small white butterfly, *Pieris rapae crucivora*. *J Neurosci.* **25**, 5935–5942.
- Arnold SJ, Duvall D (1994) Animal mating systems: a synthesis based on sexual selection theory. *Am. Nat.* **143**, 317-348.
- Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol.*, **47**, 817–844.
- Badyaev AV, Martin TE (2000) Sexual dimorphism in relation to current selection in the house finch. *Evolution* **54**, 987–997.
- Badyaev AV (2002) Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol. Evol.* **17**, 369-378.
- Batemann AJ (1948) Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349-368.
- Bauerfield SS, Fischer K (2005) Effects of food stress and density in different life stages on reproduction in a butterfly. *Oikos* **111**, 514-524
- Bedhomme S, Chippindale AK (2007) Irreconcilable differences: when sexual dimorphism fails to resolve sexual conflict. In: *Sex, size and gender roles: evolutionary studies of sexual size dimorphism* (D.J. Fairbairn, W.U. Blanckenhorn, & T. Székely, eds.), pp. 185–194. Oxford University Press, Oxford.

- Bergman M, Gotthard K, Wiklund C (2011) Mate acquisition by females in a butterfly: the effects of mating status and age on female mate-locating behaviour. *Anim. Behav.* **81**, 225-229.
- Bergström J, Wiklund C (2002) Effects of size and nuptial gifts on butterfly reproduction: Can females compensate for a smaller size through male-derived nutrients? *Behav. Ecol. Sociobiol.* **52**, 296-302.
- Bernays EA, Chapman RF (1994) *Chemicals in plants. In: Host-Plant Selection by Phytophagous Insects.* pp. 14-60. Chapman & Hall, New York.
- Bernays EA (1998) Evolution of feeding behavior in insect herbivores. *BioScience*, **48**, 35-44.
- Bissoondath CJ, Wiklund C (1995) Protein content of spermatophores in relation to monandry/polyandry in butterflies. *Behav. Ecol. Sociobiol.* **37**, 365-371.
- Bissoondath CJ, Wiklund C (1996) Effect of male mating history and body size on ejaculate size and quality in two polyandrous butterflies, *Pieris napi* and *Pieris rapae* (Lepidoptera: Pieridae). *Funct. Ecol.* **10**, 457-464.
- Blanckenhorn WU, Dixon AFG, Fairbairn DJ, Foellmer MW, Gilbert P, van der Linde K, Meier R, Nylin S, Pitnick S, Schoff C, Signorelli M, Teder T, Wiklund C (2007) Proximate causes of Rensch's rule: does sexual size dimorphism in arthropods result from sex differences in development time? *Am. Nat.* **169**, 245-257.
- Boggs CL (1981) Nutritional and life-history determinants of resource allocation in holometabolous insects. *Am. Nat.* **111**, 692-709
- Boggs CL (1995) Male nuptial gifts: Phenotypic consequences and evolutionary implications. In: Leather SR, Hardie J (eds) *Insect Reproduction*. CRC Press, Boca Raton, pp 215-242
- Boggs CL (2009) Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology*, **23**, 27-37.
- Boggs CL, Freeman KD (2005) Larval food limitation in butterflies: Effects on adult resource allocation and fitness. *Oecol.* **144**, 353-361.
- Boggs CL, Gilbert LE (1979) Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. *Science* **206**, 83-84.
- Boggs CL, Ross CL (1993) The effect of adult food limitation on life history traits in *Speyria mormonia* (Lepidoptera: Nymphalidae). *Ecology* **74**, 433-441.
- Boggs CL, Watt WB (1981) Population structure of pierid butterflies. IV. Genetic and physiological investment in offspring by male *Colias*. *Oecol.* **50**, 320-324.
- Bonduriansky R (2001) The evolution of male mate choice in insects: A synthesis of ideas and evidence. *Biol Rev Camb Phil Soc* **76**, 305-339.

- Bonduriansky R (2007) The evolution of condition-dependent sexual dimorphism. *Am. Nat.* **169**, 9–19
- Bonduriansky R (2009) Reappraising sexual coevolution and the sex-roles. *PLoS Biology* **7**, e1000255.
- Bradford M (1976) A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Bioch.* **72**, 248-254.
- Burley N (1977) Parental investment, mate choice, and mate quality. *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3476-3479.
- Butlin RK, Woodhatch CW, Hewitt GM (1987) Male spermatophore investment increases female fecundity in a grasshopper. *Evolution* **41**, 221-225
- Byrne PG, Rice WR (2006) Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*. *Proc R Soc Lond B.* **273**, 917–922.
- Chapman RF (1998) *The insects: structure and function*, 4th ed. Cambridge University Press, UK.
- Chen Y, Lin L, Wang C, Yeh C, Hwang S (2004) Response of two *Pieris* (Lepidoptera: Pieridae) species to fertilization of a host plant. *Zoological Studies*, **43**, 778-786.
- Chenoweth SF, Doughty P, Kokko H (2006) Can non-directional male preferences facilitate honest female ornamentation? *Ecology Letters* **9**, 179-184.
- Chenoweth SF, Petfield D, Doughty P, Blows MW (2007) Male choice generates stabilizing sexual selection on a female fecundity correlate. *J. Evol. Biol.* **20**, 1745–1750.
- Chippindale AK, Gibson JR, Rice WR (2001) Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *PNAS.* **98**, 1671-1675.
- Clarebrough C, Mira A, Raubenheimer D (2000) Sex-specific differences in nitrogen intake and investment by feral and laboratory-cultural cockroaches. *J Insect Physiol* **46**, 677-684
- Clutton-Brock T (2009) Sexual selection in females. *Anim Behav.* **77**, 3–11.
- Collins NC (1980) Developmental responses to food limitation as indicators of environmental conditions for *Ephydra cinerea* Jones (Diptera). *Ecology* **61**, 650-661
- Conner J, Via S (1992) Natural selection on body size in *Tribolium*: possible genetic constraints on adaptive evolution. *Heredity* **69**, 73-83.

- Conner JK, Hartl DL (2004) A primer of ecological genetics. Sinauer Associates, Inc, Sunderland, Massachusetts USA.
- Cook PA, Wedell N (1996) Ejaculate dynamics in butterflies: a strategy for maximizing fertilization success? *Proc R Soc Lond B.* **263**, 1047-1051.
- Cortese MD, Norry FM, Piccinali R, Hasson E (2002) Direct and correlated responses to artificial selection on developmental time and wing length in *Drosophila buzzatii*. *Evolution* **56**, 2541-2547.
- Cotton S, Fowler K, Pomiankowski A (2004) Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc R Soc Lond B.* **271**, 771–783
- Cotton S, Small J, Pomiankowski A (2006) Sexual selection and condition-dependent mate preferences. *Current Biology* **16**, R755–R765.
- Cox RM, Calsbeek R (2009) Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *Am Nat.* **173**: 176-187.
- Darwin C (1859) On the origin of species. From so simple a beginning (Ed Edward O. Wilson), 1st edition, W.W. Norton and Company, Inc. NY
- Darwin C (1874) The descent of man and selection in relation to sex. From so simple a beginning (Ed Edward O. Wilson), 1st edition, W.W. Norton and Company, Inc. NY.
- Davidowitz G, Roff DA, Nijhout HF (2005) A physiological perspective on the response of body size and development time to simultaneous directional selection. *Integrat Comparat Biol.* **45**, 525-531.
- De Block M, Stoks R (2005) Fitness effects from egg to reproduction: bridging the life history transition, *Ecology*, **86**, 185–197.
- De Block M, Stoks R (2008) Compensatory growth and oxidative stress in a damselfly. *Proc R Soc Lond B.* **275**, 781–785.
- Delph LF, Gehring JL, Frey FM, Artanz AM, Levri M (2004) Genetic constraints on floral evolution in a sexually dimorphic plant revealed by artificial selection. *Evolution* **58**, 1936- 1946.
- Dewsbury DA (1982) Ejaculate cost and male choice. *Am Nat.* **119**, 601–610
- Doutrelant C, Gregoire A, Grnac N, Gomez D, Lambrechts MM, Perret P (2008) Female colouration indicates female reproductive capacity in blue tits. *J. Evol. Biol.* **21**, 226–233.

- Drummond BB (1984) Multiple mating and sperm competition in the Lepidoptera. In: Smith RL (ed) *Sperm competition and the evolution of animal mating systems*. Academic Press, London, pp 547–572
- Edward DA, Chapman T (2011) The evolution and significance of male mate choice. *Trends Ecol. Evol.* **26**, 647–654.
- Eguchi E, Watanabe K, Hariyama T, Yamamoto K (1982) A comparison of electrophysiologically determined spectral responses in 35 species of Lepidoptera. *J. Insect Physiol.* **28**, 675–682.
- Ellers J, Boggs CL (2003) The evolution of wing color: male mate choice opposes adaptive wing color divergence in *Colias* butterflies. *Evolution* **57**, 1100–1106.
- Elser JJ, Fagan WF, Denno RF et al. (2000) Nutritional constraints in terrestrial and freshwater food webs. *Nature* **408**, 578–580
- Englert DC, Bell AE (1969) Components of growth in genetically diverse populations of *Tribolium castaneum*. *Can. J. Genet. Cytol.* **11**, 896–907.
- Engqvist L, Dekomien G, Lippmann T, Epplen JT, Sauer KP, (2007) Sperm transfer and paternity in the scorpionfly *Panorpa cognata*: large variance in traits favoured by post-copulatory episodes of sexual selection. *Evol. Ecol.* **21**, 801–816.
- Evans JP (2010) Quantitative genetic evidence that males trade attractiveness for ejaculate quality in guppies. *Proc. R. Soc. B.* **277**, 3195 – 3201.
- Fairbairn DJ (2007a) Sexual dimorphism in the water strider, *Aquarius remigis*: a case study of adaptation in response to sexually antagonistic selection. In: *Sex, size and gender roles: evolutionary studies of sexual size dimorphism* (D.J. Fairbairn, W.U. Blanckenhorn, & T. Székely, eds.), pp. 97–105. Oxford University Press, Oxford.
- Fairbairn DJ (2007b) Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. *Annu Rev Ecol Syst.* **28**, 659–687.
- Fairbairn DJ, Blanckenhorn WU, Székely T eds. (2007) *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. Oxford University Press, Oxford.
- Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*, 4th Edition, Longman, Essex, England.
- Fedina TY, Lewis SM (2006) Female influence over offspring paternity in the red flour beetle *Tribolium castaneum*. *Proc R Soc Lond B.* **271**, 1393–1399
- Fedina TY, Lewis SM (2008) An integrative view of sexual selection in *Tribolium* flour beetles. *Biol Rev Camb Philos Soc.* **83**, 151–157.

Fellous S, Lazzaro BP (2010) Larval food quality affects adult (but not larval) immune gene expression independent of effects on general condition. *Molec. Ecol.* **19**, 1462-1468.

Fitzpatrick S, Berglund A, Rosenqvist G (1995) Ornaments or offspring-costs to reproductive success restrict sexual selection processes. *Biol. J. Linn. Societ.* **55**, 251-260.

Foote CJ, Brown GS, Hawryshyn CW (2004) Female colour and male choice in sockeye salmon: implications for the phenotypic convergence of anadromous and nonanadromous morphs. *Anim. Behav.* **67**, 69-83.

Forero MG, Tella JL, Donazar JA, Blanco G, Bertellotti M, Ceballos O (2001) Phenotypic assortative mating and within-pair sexual dimorphism and its influence on breeding success and offspring quality in Magellanic penguins. *Canad. J. Zool.* **79**, 1414-1422.

Fox CW, Stillwell RC, Moya-Laraño J (2007) Variation in selection, phenotypic plasticity, and the ecology of sexual size dimorphism in two seed-feeding beetles. In: *Sex, size and gender roles: evolutionary studies of sexual size dimorphism* (D.J. Fairbairn, W.U. Blanckenhorn, & T. Székely, eds.), pp. 88-96. Oxford University Press, Oxford.

Frankino WA (2009) Experimental approaches to studying the evolution of morphological allometries: The shape of things to come. Invited submission in: *Experimental Evolution: Concepts, Methods, and Applications*, T. Garland and M. Rose, eds. University of California Press.

Frankino WA, Zwaan BJ, Stern DL, Brakefield PM (2007) Internal and external constraints in the evolution of a forewing-hindwing allometry. *Evolution* **61**, 2958-2970.

Gowaty PA, B. Steinichen, WW. Anderson. 2003. Indiscriminate females and choosy males: Within and between species variation in *Drosophila*. *Evolution* **57**, 2037-2035.

Gotthard K, Nylin S, Wiklund C (1994) Adaptive variation in growth rate-life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. *Oecol.* **99**, 281-289.

Gwynne DT (2001) *Katydid and bush-cricket: reproductive behavior and evolution of the Tettigoniidae*. Cornell University Press, New York

Gwynne DT (2004) Sexual differences in response to larval food stress in two nuptial feeding orthopterans-implications for sexual selection. *Oikos* **105**, 619-625

Gwynne DT (2008) Sexual conflict over nuptial gifts in insects. *Annu Rev Entomol* **53**, 83-101

- Hardling R, Kokko H (2005) The evolution of prudent choice. *Evol Ecol Res.* **7**, 697–715.
- Hedrick AV, Temeles EJ (1989) The evolution of sexual dimorphism in animals: hypotheses and tests. *Trends Ecol. Evol.* **4**, 136–138.
- Holveck MJ, Geberzahn N, Riebel K (2011) An Experimental Test of Condition-Dependent Male and Female Mate Choice in Zebra Finches. *PLoS ONE*, **6**, e23974.
- Holveck MJ, Geberzahn N, Riebel K (2011) An experimental test of condition-dependent male and female mate choice in zebra finches. *PLoS ONE* **6**, e23974.
- Howe RW (1968) A further consideration of the heterogeneity of the developmental period of *Tribolium castaneum* (Herbst) (Col., Tenebrionidae) in constant environmental conditions. *J. stored Prod. Res.* **4**, 221–231.
- Huberty AF, Denno RF (2006) Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent life-history strategies. *Oecol.* **149**, 444–455.
- Hughes C (1998) Integrating molecular techniques with field methods in studies of social behavior: a revolution result. *Ecology* **79**, 383–399.
- Hunt J, Brooks R, Jennions MD et al. (2004) High-quality male field crickets invest heavily in sexual display but die young. *Nature* **432**, 1024–1027
- Hwang SY, Liu CH, Shen TC (2008) Effects of plant nutrient availability and host plant species on the performance of two *Pieris* butterflies (Lepidoptera: Pieridae). *Biochem. System. Ecol.*, **36**, 505–513.
- Jannot JE, Bruneau E, Wissinger SA (2007) Effects of larval energetic resources on life history and adult allocation patterns in a caddisfly (Trichoptera: Phryganeidae). *Ecol. Entomol.* **32**, 376–383
- Johnstone RA, Reynolds JD, Deutsch JC (1996) Mutual mate choice and sex differences in choosiness. *Evolution.* **50**, 1382–1391.
- Kaitala A, Wiklund C (1994) Polyandrous female butterflies forage for matings. *Behav Ecol Sociobiol.* **35**:385–388
- Kandori I, Ohsaki N (1996) Male mating behavior in relation to spermatophore transfer in the white cabbage butterfly. *Res. Pop. Ecol.* **38**, 225–230.
- Karlsson B (1995) Resource allocation and mating systems in butterflies. *Evolution* **49**, 955–961
- Karlsson B (1996) Male reproductive reserves in relation to mating system in butterflies: a comparative study. *Proc. R Soc. Lond. B.* **263**, 187–192

- Karlsson B (1998) Nuptial gifts, resource budgets, and reproductive output in a polyandrous butterfly. *Ecology* **79**, 2931-2940
- Karlsson B, Leimar O, Wiklund C (1997) Unpredictable environments, nuptial gifts and the evolution of sexual size dimorphism in insects: an experiment. *Proc. R Soc. Lond. B.* **264**, 475-479
- Kayser H (1985) Pigments. Comprehensive insect physiology, biochemistry and pharmacology. (eds G.A Kerkut, & L.I. Gilbert), pp. 367–415, Pergamon, New York.
- Kemp DJ, Rutowski RL (2007) Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. *Evolution* **61**, 168-183.
- Klaus SP, Fitzsimmons LP, Trevor EP, Bertram SM (2011) Song and sperm in Crickets: A trade-off between pre- and post-copulatory traits or phenotype-linked fertility? *Ethology* **117**, 154–162
- Knüttel H, Fiedler K (2001) Host-plant-derived variation in ultraviolet wing patterns influences mate selection by male butterflies. *J. Exp. Biol.* **204**, 2447–2459.
- Kokko H, Johnstone RA (2002) Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. *Philos Trans R Soc London B.* **357**, 319-330.
- Kokko H, Monaghan P (2001) Predicting the direction of sexual selection. *Ecol. Lett.* **4**, 159–165.
- Kokko H (1998) Should advertising parental care be honest *Proc R Soc Lond B.* **265**, 1871-1878.
- Koncagül S, Berger PJ (2006) Response to selection for increased pupa weight in *Tribolium castaneum* in different environments. *J. Agric. Fac.* **10**: 1-10
- Kraaijeveld K, Gregurke J, Hall C, Komdeur J, Mulder RA (2004) Mutual ornamentation, sexual selection, and social dominance in the black swan. *Behav Ecol.* **15**, 380-389
- Kraaijeveld K, Kraaijeveld-Smit FJL, Komdeur J (2007) The evolution of mutual ornamentation. *Anim. Behav.* **62**, 1021-1026.
- LaMunyon CW, Eisner T (1994) Spermatophore size as determinant of paternity in an arctiid moth (*Utetheisa ornatrix*). *Proc Nat Acad Sci (USA)* **91**, 7081-7084
- Lande R (1980) Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* **34**, 292–305.
- Lande R (1987) Genetic correlations between the sexes in the evolution of sexual dimorphism and mating preferences. In: *Sexual Selection: Testing the Alternatives*

(Bradbury, J.W. and Andersson, M.B., eds.) pp. 83-94. John Wiley & Sons, Chichester.

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* **37**, 1210-1226.

LeBas NR, Hockham LR, Ritchie MG (2003) Nonlinear and correlational sexual selection on 'honest' female ornamentation. *Proc. R. Soc. Lond. B* **270**, 2159–2164.

Lee T (2007) Female ornamentation and intrasexual competition in the convict cichlid fish (*Archocentrus nigrofasciatus*). PhD thesis, UCLA.

Leimar O, Karlsson B, Wiklund C (1994) Unpredictable food and sexual size dimorphism in insects. *Proc R Soc Lond B*. **258**,121-125

Lewis S, South A (2012) Evolution of animal nuptial gifts. *Adv. Study. Behav.* **44**, 53-97

Lewis S, South A, Al-Wathiqui N, Burns R (2011) Quick guide: Nuptial gifts. *Curr. Biol.* **21**, 644-645.

Lewis SM, South A (2012) The evolution of animal nuptial gifts. *Advances in the Study of Behavior* **44**, 53-97.

Lewis SM, Tigreros N, Fedina T, Ming QL (2012) Genetic and nutritional effects on male traits and reproductive performance in *Tribolium* flour beetles. *J. Evol. Biol.* **25**, 438–451.

Lewis Z, Sasaki H, Miyatake T (2011) Sex starved: do resource-limited males ensure fertilization success at the expense of precopulatory mating success? *Anim.l Behav.* **81**, 579-583.

Magwere T, Chapman T, Partridge L (2004) Sex differences in the effect of dietary restriction on life span and mortality rates in female and male *Drosophila melanogaster*. *J Gerontol.* **59A**, 3-9

Maklakov AA, Simpson SJ, Zajitschek F et al. (2008) Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Curr. Biol.* **18**, 1062–1066

Mank JE (2009) Sex chromosomes and the evolution of sexual dimorphism: lessons from the genome. *Am Nat.* **173**, 141–150.

Marshall LD (1982) Male nutrient investment in the Lepidoptera: what nutrients should males invest? *Am. Nat.* **120**, 273-279.

Mattson WJ (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**,119-161.

McDaniel SF (2005) Genetic correlations do not constrain the evolution of sexual dimorphism in the Moss *Ceratodon purpureus*. *Evolution* **59**, 2353-2361.

- McGraw LA, Fiumera AC, Ramakrishnan M, Madhavarapu S, Clark AG, Wolfner MF (2007) Larval rearing environment affects several post-copulatory traits in *Drosophila melanogaster*. *Biology letters*, **3**, 607-610.
- McNamara JM, Houston AI (1996) State-dependent life histories. *Nature* **380**, 215–221.
- Meagher TR (1992) The quantitative genetics of sexual dimorphism in *Silene latifolia* (Caryophyllaceae). I. Genetic Variation. *Evolution* **46**, 445-457.
- Messina FJ (2004) Predictable modification of body size and competitive ability following a host shift by a seed beetle. *Evolution* **58**, 2788-2797.
- Moczek AP (1998) Horn polyphenism in the beetle *Onthophagus taurus*: larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. *Behav. Ecol.* **9**, 636-641
- Morales J, Velando A, Torres R (2009) Fecundity compromises attractiveness when pigments are scarce. *Behavioral Ecology*, **20**, 117–123.
- Moran NA (1994) Adaptation and Constraint in the Complex Life Cycles of Animals. *Annual Review of Ecology and Systematics*, **25**, 573-600.
- Moreau J, Benrey B, Thiéry D (2006) Assessing larval food quality for phytophagous insects: are facts as simple as it appears? *Functional Ecology*, **20**, 592–600.
- Morehouse NI, Rutowski RL (2010) In the eyes of the beholders: Female choice and avian predation risk associated with an exaggerated male butterfly color. *Amer. Nat.* **176**, 768-784
- Morehouse NI (2009) Sexually selected male color ornament advertises direct and indirect benefits in a butterfly. Limiting nutrients, female choice, and male color. PhD thesis, Arizona State University.
- Morehouse NI, Vukusic P, Rutowski R (2007) Pterin pigment granules are responsible for both broadband light scattering and wavelength selective absorption in the wing scales of pierid butterflies. *Proc. Roy. Soc. B.* **274**, 359–366.
- Morehouse NI (2009) Sexually selected male color ornament advertises direct and indirect benefits in a butterfly. Limiting nutrients, female choice, and male color. PhD thesis, Arizona State University
- Morehouse NI, Nakazawa T, Booher CM et al. (2010) Sex in a material world: Why the study of sexual reproduction and sex-specific traits should become more nutritionally-explicit. *Oikos* **119**, 766-778.
- Morehouse NI, Rutowski RL (2010a) In the eyes of the beholders: Female choice and avian predation risk associated with an exaggerated male butterfly color. *Amer. Nat.* **176**, 768-784.

- Morehouse NI, Rutowski RL (2010b) Developmental responses to variable diet composition in the cabbage white butterfly, *Pieris rapae*: the role of nitrogen, carbohydrates and genotype. *Oikos* **199**, 636-645
- Morehouse NI, Vukusic P, Rutowski R (2007) Pterin pigment granules are responsible for both broadband light scattering and wavelength selective absorption in the wing scales of pierid butterflies. *Proc. R Soc. Lond. B.* **274**, 359-366
- Morehouse NI, Rutowski RL (2010b) In the eyes of the beholders: Choice and avian predation risk associated with an exaggerated male butterfly color. *The American Naturalist.* **176**, 768-784.
- Morehouse NI, Rutowski RL (2010a) Developmental responses to variable diet composition in the cabbage white butterfly, *Pieris rapae*: the role of nitrogen, carbohydrates and genotype. *Oikos*, **119**, 636-645.
- Morehouse NI (2009) Sexually selected male color ornament advertises direct and indirect benefits in a butterfly. *Limiting nutrients, female choice, and male color*. PhD thesis, Arizona State University.
- Morehouse NI, Nakazawa T, Booher CM, Jeyasingh PD, Hall MD (2010) Sex in a material world: Why the study of sexual reproduction and sex-specific traits should become more nutritionally-explicit. *Oikos*, **119**, 766-778.
- Mullins D, Keil C, White RH (1992) Maternal and paternal nitrogen investment in *Blattella germanica* (Dictyoptera: Blattellidae). *J. Exp. Biol.* **162**, 55-72
- Moczek AP, Nijhout HF (2004) Tradeoffs during the development of primary and secondary sexual traits in a dimorphic beetle. *The American Naturalist* **163**, 184-191
- Nandy B, Joshi A, Ali ZS, Sen S, Prasad NG (2012) Degree of adaptive male mate choice is positively correlated with female quality variance. *Sci Rep.* **2**, 447.
- Nijhout HF, Roff DA, Davidowitz G (2010) Conflicting processes in the evolution of body size and development time. *Phil. Trans. R. Soc. B* **365**, 567-575.
- Nijhout HF, Davidowitz G, Roff DA (2006) A quantitative analysis of the mechanism that controls body size in *Manduca sexta*. *J Biol.* **5**:16.
- Nordeide JT, Kekäläinen J, Janhunen M, Kortet R (2013) Female ornaments revisited-are they correlated with offspring quality? *J of Animal Ecology* **82**, 26-38.
- Nylin S, Gotthard K (1998) Plasticity in life history-traits. *Annu. Rev. Entomol.* **43**, 63-83
- O'Brien DM, Boggs CL, Fogel ML (2004) Making eggs from nectar: The role of life history and dietary carbon turnover in butterfly reproductive resource allocation. *Oikos* **105**, 279-291

- Obara Y, Majerus MEN (2000) Initial mate recognition in the British cabbage butterfly, *Pieris rapae rapae*. *Zool. Sci.* **17**,725-730
- Obara Y, Ozawa G, Fukano Y et al. (2008) Mate preference in males of the cabbage butterfly, *Pieris rapae crucivora*, changes seasonally with the change in female UV color. *Zool. Sci.* **25**.1-5.
- Obara Y, Majerus ME N (2000) Initial mate recognition in the British cabbage butterfly, *Pieris rapae rapae*. *Zool Sci* **17**, 725-730.
- Obara Y, Koshitaka H, Arikawa K (2008) Better mate in the shade: enhancement of male mating behaviour in the cabbage butterfly, *Pieris rapae crucivora*, in a UV-rich environment. *J. Experim. Biol.* **211**, 3698-3702.
- Obara Y, Ozawa G, Fukano Y, Watanabe K, Satoh T (2008) Mate preference in males of the cabbage butterfly, *Pieris rapae crucivora*, changes seasonally with the change in female UV color. *Zoological Science*, **25**, 1-5.
- Obika M, Bagnara JT (1964) Pteridines as pigments in amphibians. *Science* **143**, 485-87.
- Owens IPF, Thompson DBA (1994) Sex differences, sex ratios and sex roles. *Proc. R. Soc. Lond. B.* **258**, 93–99.
- Parker GA (1983) Mate quality and mating decisions. In *Mate choice* (ed. P. Bateson). p. 141-164 Cambridge University Press.
- Partridge L, Fowler K (1993) Responses and correlated responses to artificial selection on thorax length in *Drosophila melanogaster*. *Evolution* **47**, 213-226.
- Partridge L, Langelan R, Fowler K, Zwaan B, French V (1999) Correlated responses to selection on body size in *Drosophila melanogaster*. *Genet. Res.* **74**, 43-54.
- Pechenick JA, Wendt DE, Jarrett JN (1998) Metamorphosis is not a new beginning: larval experience influences juvenile performance, *BioScience* **48**, 901–910.
- Pierce NE, Berry A (2011) The herbivore's dilemma: never enough nitrogen. In: *In the light of evolution: essays from the laboratory and field*. Edit. Losos J.
- Pijpe J, Fischer K, Brakefield PM, Zwaan BJ (2006) Consequences of artificial selection on pre-adult development for adult lifespan under benign conditions in the butterfly *Bicyclus anynana*. *Mechanisms of ageing and development*, **127**, 802-807.
- Poissant J, Wilson AJ, Coltman DW (2010) Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlation. *Evolution* **64**, 97-107.
- Preston BT, Stevenson IR, Pemberton JM, Coltman DW, Wilson K (2005) Male mate choice influences female promiscuity in Soay sheep. *Proc. Biol. Sci.* **272**, 365-373.

- Preziosi RF, Fairbairn DJ (2000) Lifetime selection on adult body size and components of body size in a waterstrider: opposing selection and maintenance of sexual size dimorphism. *Evolution* **54**, 558-566.
- Raubenheimer D, Simpson SJ, Mayntz D (2009) Nutrition, ecology and nutritional ecology: toward an integrated framework. *Funct. Ecol.*, **23**, 4–16.
- Real L (1990) Search theory and mate choice. I. Models of single- sex discrimination. *Am. Nat.* **136**, 376-404.
- Reeve JP, Fairbairn DJ (1996) Sexual size dimorphism as a correlated response to selection on body size: an empirical test of the quantitative genetic model. *Evolution* **50**, 1927-1938.
- Reeve JP, Fairbairn DJ (2001) Predicting the evolution of sexual size dimorphism. *J. Evol. Biol.* **14**, 244-254.
- Ringo J (1996) Sexual receptivity in insects. *Annu. Rev. Entomol.* **41**, 473-494
- Rivero A, Giron D, Casas J (2001) Lifetime allocation of juvenile and adult nutritional resources to egg production in a holometabolous insect. *Proceedings of the Royal Society of London. B.* **268**, 1231–1237.
- Roff DA, Fairbairn DJ (2007) The evolution of trade-offs: where are we? *J. Evol. Biol.* **20**, 433–447.
- Roff DA (2002) *Life History Evolution*. Sinauer Associates, Sunderland, MA.
- Roff DA (1997) *Evolutionary Quantitative Genetics*. Chapman and Hall, New York.
- Roff DA (2000) Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. *J. Evol. Biol.* **13**, 434-445.
- Rolff J, Armitage SAO, Coltman DW (2005) Genetic constraints and sexual dimorphism in immune defense. *Evolution* **59**, 1844-1850.
- Rowe L, Ludwig D (1991) Size and timing of metamorphosis in complex life histories, time constraints and variation, *Ecology*, **72**, 413–427.
- Rutowski RL (1982) Mate Choice and lepidopteran mating behavior. *The Florida Entomol.* **65**, 72-82.
- Rutowski RL (1980) Courtship solicitation by females of the Checkered White butterfly, *Pieris protodice*. *Behav. Ecol. Sociob.* **7**, 113-117.
- Rutowski RL (1981) Sexual discrimination using visual cues in the checkered white butterfly (*Pieris protodice*). *Zeitschrift für Tierpsychologie.* **55**, 325-334.

- Rutowski RL, Macedonia JM, Morehouse N, Taylor-Taft L (2005) Pterin pigments amplify iridescent ultraviolet signal in males of the orange sulphur butterfly, *Colias eurytheme*. *Proc. Biol. Sci.* **272**, 2329–2335.
- Saether SA (2002) Female calls in lek-mating birds: indirect mate choice, female competition for mates, or direct mate choice? *Behav. Ecol.* **13**, 344-352.
- Sappington TW, Taylor OR (1990) Disruptive sexual selection in *Colias eurytheme* butterflies. *Proc. natn. Acad. Sci. U.S.A.* **87**, 6132–613
- Scott JA (1973) Lifespan of butterflies. *J. Res. Lep.* **12**, 225-230.
- Scriber JM, Slansky F Jr (1981). Nutritional ecology of immature insects. *Ann. Rev. Entomol.* **26**,183–211.
- Servedio MR (2007) Male versus female mate choice: sexual selection and the evolution of species recognition via reinforcement. *Evolution.* **61**, 2772–2789.
- Setchell JM, Wickings EJ (2002) Mate choice in male mandrills (*Mandrillus sphinx*). *Ethology* **12**, 91-99.
- Shapiro AM (1970) The role of sexual behavior in density-related dispersal of pierid butterflies. *Amer. Nat.* **104**, 367-372
- Shapiro J P, Ferkovich SM (2002) Yolk protein immunoassays (YP-ELISA) to assess diet and reproductive quality of mass-reared *Orius insidiosus* (Heteroptera: Anthocoridae). *J.Econ. Entomol.* **95**, 927–935
- Shingleton AW, Frankino WA, Flatt T, Nijhout HF, Emlen DJ (2007) Size and shape: the developmental regulation of static allometry in insects. *BioEssays* 29: 536–548.
- Sibly R, Calow P (1986) Why breeding earlier is always worthwhile. *J. Theor. Biol.* **123**, 311-319.
- Silberglied RE, Taylor OR (1978) Ultraviolet reflection and its behavioral role in the courtship of the sulphur butterflies *Colias eurytheme* and *C. philodice* (Lepidoptera, Pieridae). *Behav. Ecol. Sociobiol.* **3**, 203–243.
- Silberglied RE (1984) Visual communication and sexual selection among butterflies. In *The biology of butterflies* (ed. R. I. Vane-Wright and P. R. Ackery) pp 207-223 Academic Press, London.
- Simmons LW, Emlen DJ (2008) No fecundity cost of female secondary sexual trait expression in the horned beetle *Onthophagus sagittarius*. *J. Evol. Biol.* **21**, 1227–1235.
- Simmons LW (2001) *Sperm competition and its evolutionary consequences in insects*. Princeton Monographs in Behavior and Ecology.

- Simmons LW, Llorens T, Schinzig M, Hosken D, Craig M (1994) Sperm competition selects for male mate choice and protandry in the bushcricket, *Requena verticalis* (Orthoptera: Tettigoniidae). *Animal Behaviour*, **47**, 117-122.
- Slansky FJr, Feeny P (1977) Stabilization of the rate of nitrogen accumulation by larvae of the cabbage white butterfly on wild and cultivated food plants. *Ecol. Monogr.* **47**, 209-228
- Slansky FJr, Rodriguez JG (1987) Nutritional ecology of insects, mites, spiders and related invertebrates: an overview. In *Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates*. (eds. F. Slansky Jr. & J. G. Rodriguez), pp. 1–69. John Wiley & Sons, London, New York.
- Slansky, F.Jr. & Feeny, P. (1977). Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecol. Monog.* **47**, 209–228.
- Sokoloff A (1977) *The Biology of Tribolium*, Vol. 3. Clarendon Press, Oxford.
- South A, SM Lewis (2012a) Determinants of reproductive success across sequential episodes of sexual selection in a firefly. *Proc. R. Soc. B.* **279**, 3201-3208.
- South A, S.M. Lewis (2012b) Effects of male ejaculate on female reproductive output and longevity in *Photinus* fireflies. *Canad. J. Zool.* **90**, 677-681.
- Stearns SC (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.
- Steffen JE, McGraw KJ (2007) Contributions of pterin and carotenoid pigments to dewlap coloration in two anole species. *Comp. Biochem. Physiol., Part B* **146**, 42-46.
- Stillwell RC, Davidowitz G (2010) Sex differences in phenotypic plasticity of a mechanism that controls body size: implications for sexual dimorphism. *Proc. R. Soc. B.* **277**, 3819-3826
- Stillwell RC, Davidowitz G (2010) A developmental perspective on the evolution of sexual size dimorphism of a moth. *Proc. R. Soc. B.* **277**, 2069-2074.
- Stillwell RC, Blanckenhorn WU, Teder T, Davidowitz G, CW Fox (2010) Sex differences in phenotypic plasticity of body size affects variation in sexual size dimorphism in insects: from physiology to evolution. *Annu. Rev. Entomol.* **55**, 227-245.
- Stoks R, De Block M, McPeck M (2006) Physiological costs of compensatory growth in a damselfly, *Ecology* **87**, 1566-1574.
- Sugawara T (1979) Stretch reception in the bursa copulatrix of the butterfly, *Pieris rapae crucivora*, and its role in behaviour. *J. Comp. Physiol. A.* **130**, 191-199.
- Suzuki Y (1979) Mating frequency in females of the small cabbage white, *Pieris rapae crucivora* Boisduval (Lepidoptera: Pieridae). *Kontyu* **47**, 335-339.

- Suzuki Y, Nakanishi A, Shima H, Yata O, Saigusa T (1977) Mating behaviour of four Japanese species of the genus *Pieris* (Lepidoptera, Pieridae). *Kontyu*, **45**, 300-313.
- Svärd L, Wiklund C (1989) Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. *Behav. Ecol. Sociobiol.* **24**, 395-402
- Teuschl Y, Reim C, Blanckenhorn, WU (2007) Correlated responses to artificial body size selection in growth, development, phenotypic plasticity and juvenile viability in yellow dung flies. *J. Evol. Biol.* **20**: 87-103.
- Thomas DB, McGoverin CM, McGraw KJ, James HF, Madden O (2013) Vibrational spectroscopic analyses of unique yellow feather pigments
- Tigreros N (2013) Linking nutrition and sexual selection across life stages in a model butterfly system. *Funct. Ecol.* **27**, 145-154.
- Tigreros N, Sass E, Lewis SM (2013). Sex-specific responses to nutrient limitation and its effects on female mating success in a gift-giving butterfly. *Evolut. Ecol.* DOI 10.1007/s10682-013-9647-x
- Tobias JA, Montgomerie R, Lyon BE (2012) The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Phil. Trans. R Soc B.* **367**, 2274-2293.
- Trivers RL (1972) Parental investment and sexual selection. In *Sexual selection and the Descent of Man, 1871–1971* (Campbell, BG, ed.), pp. 136–179, Aldine.
- Trivers RL (1985) *Social evolution.*: Benjamin/Cummings pub. Menlo Park CA.
- Tudor MS, Morris MR (2009) Variation in male mate preference for female size in the swordtail *Xiphophorus malinche*. *Behav.* **146**, 727–740.
- Vahed K (1998) The function of nuptial feeding in insects: a review of empirical studies. *Biol. Rev.* **73**, 43-78.
- Van Noordwijk AJ, De Jong G (1986) Acquisition and allocation of resources – their Influence on variation in life-history tactics. *Am. Nat.* **128**, 137–142.
- Via S, Conner J (1995) Evolution in heterogeneous environments: genetic variability within and across different grains in *Tribolium castaneum*. *Heredity* **74**: 80-90.
- Wall R, Wearmouth VJ, Smith KE (2002) Reproductive allocation by the blow fly *Lucilia sericata* in response to protein limitation. *Physiol. Entomol.* **27**, 267–274.
- Watanabe M, Ando S (1993) Influence of mating frequency on lifetime fecundity in wild females of the small white *Pieris rapae* (Lepidoptera, Pieridae). *Jap. J. Entomol.* **61**, 691-696.

- Watt WB, Chew FS, Snyder LRG, Watt AG, Rothschild DE (1977) Population structure of *pierid* butterflies. I. Numbers and movements of some montane *Colias* species. *Oecol.* **27**, 1–22.
- Wedell N, Cook PA (1998) Determinants of paternity in a butterfly. *Proc. R. Soc. B.* **265**, 625-630.
- Wedell N, Cook PA (1999) Strategic sperm allocation in the small white butterfly *Pieris rapae* (Lepidoptera:Pieridae). *Function. Ecol.* **13**, 85-93.
- Wedell N (2005) Sperm competition in butterflies and moths in Fellowes MDE, Holloway GJ, Rolff J (eds.) *Insect Evolutionary Ecology*, CABI Publishing, 49-81.
- Weiss SL (2006) Female-specific color is a signal of quality in the striped plateau lizards (*Sceloporus virgatus*). *Behav. Ecol.* **17**, 726-732.
- Weiss SL, Kennedy EA, Safran RJ, McGraw KJ (2011) Pterin-based ornamental coloration predicts yolk antioxidant levels in female striped plateau lizards (*Sceloporus virgatus*) *J. Anim. Eco.* **80**, 519-527.
- Wheeler D (1996) The role of nourishment in oogenesis. *Annu. Rev. Entomol.* **41**, 407-431.
- White TCR (1984) The availability of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecol.* **3**, 71–86.
- Wijnen B, Leertouwer HL, Stavenga DG (2007) Colors and pterin pigmentation of pierid butterfly wings. *J. Insect. physiol.* **53**, 1206-17.
- Wiklund C, Wickman PO, Nylin S (1992) A sex difference in the propensity to enter direct/diapause development: a result of selection for protandry. *Evolution*, **46**, 519-528.
- Wilkinson GS (1993) Artificial sexual selection alters allometry in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). *Genet. Res., Camb.* **62**, 213-222.
- Wolfson JL (1982) Developmental responses of *Pieris rapae* and *Spodoptera eridania* to environmentally induced variation in *Brassicacn igr*. *Environ. Entomol.*, **11**, 207-213.
- Yasui Y (1998) The ‘genetic benefits’ of female multiple mating reconsidered *TREE*. **13**, 246–250.
- Yildizhan S, van Loon J, Sramkova A, Ayasse M, Arsene C, ten Broeke C, Schulz S (2009) Aphrodisiac pheromones from the wings of the small cabbage white and large cabbage white butterflies, *Pieris rapae* and *Pieris brassicae*. *Chembiochem*, **10**, 1666-77.

