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Genetic and phenotypic variation in resource acquisition and allocation: testing a fundamental model for life-history evolution

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Genetic and Phenotypic Variation in Resource Acquisition and Allocation: Testing a Fundamental Model for Life-History Evolution

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

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Evolution, Ecology, and Organismal Biology

by

Elizabeth Griep King

June 2010

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To my parents

ABSTRACT OF THE DISSERTATION

Genetic and Phenotypic Variation in Resource Acquisition and Allocation: Testing a Fundamental Model for Life-History Evolution

by

Elizabeth Griep King

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, June 2010
Dr. Daphne J. Fairbairn, Co-Chairperson
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One of the major goals of research in evolutionary biology is explaining the biological diversity of organismal life histories. One major explanation of this diversity is that limited resources acquired by an organism must be allocated to various life processes, leading to trade-offs between these processes. In this dissertation, I use a model system—the trade-off between flight capability and reproduction in wing dimorphic insects—to investigate variation in the acquisition of resources and its subsequent allocation to competing processes.

I showed that the pattern of allocation varies in response to temperature in the water strider, *Aquarius remigis* and that this response has the potential to evolve. In addition, I used a quantitative genetic simulation model to show that the optimal allocation strategy depends on the predictability of resource levels across time. Specifically, this model predicts completely different responses of allocation to resource level variation in environments with low versus high predictability of resource levels across time.

Combining physiological assays with a quantitative genetic breeding experiment, I measured genetic and phenotypic variation in acquisition and allocation in and across several different resource environments in the wing dimorphic cricket, *Gryllus firmus*. I found a significant genetic variance for both acquisition and allocation, but I also found that independent evolution in different resource environments is constrained. I show that when allocation is measured independently of acquisition, there is evidence for the trade-off even when it may be obscured by variance in acquisition when measured only as the correlation between functions.

Using this data set, I also tested the assumptions and predictions of a theoretical model (the Y model) that predicts patterns of variation in trade-offs. An assumption of the Y model, that acquisition and allocation will be independent of one another, was not supported. However, despite this result, I found strong support for the predictions of the Y model in this system, demonstrating the Y model is robust to violations of the assumption of independence. This study is the first to use estimates of acquisition and allocation to directly test the Y model.

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Introduction

Organisms have a finite amount of energy to devote to basic life processes such as reproduction and dispersal. They must then ‘decide’ how to allocate this energy to competing structures and functions, leading to “trade-offs” between these processes (Gadgil and Bossert 1970; Bell and Koufopanou 1985; Reznick 1985; Partridge and Harvey 1988; Roff 1992; Stearns 1989; Stearns 1992; Roff 2002). Resource-based trade-offs are expected to produce negative correlations between the competing traits, and models of life history evolution assume these trade-offs constrain the evolutionary trajectories of life history traits. For example, evolutionary models often assume a trade-off between offspring number and offspring size because individuals that allocate fewer resources to each offspring will be able to produce more offspring than individuals that allocate a lot to each offspring (e.g., Smith and Fretwell 1974; Stearns 1992; Roff 1992, 2002). To test the generality of this pattern, Charnov and Ernest (2006) use comparative data across mammal species to show the expected negative relationship between offspring size and offspring number (Figure 0.1). This trade-off is hypothesized to constrain evolution in the direction of producing many, large, well-provisioned offspring, and these types of trade-offs are assumed to be ubiquitous across species. Understanding how these trade-offs shape different allocation strategies among populations and species is central to understanding biological diversity.

Despite the assumption that trade-offs between life history traits will be ubiquitous, trade-off patterns often depart significantly from this expectation (for reviews see Bell and

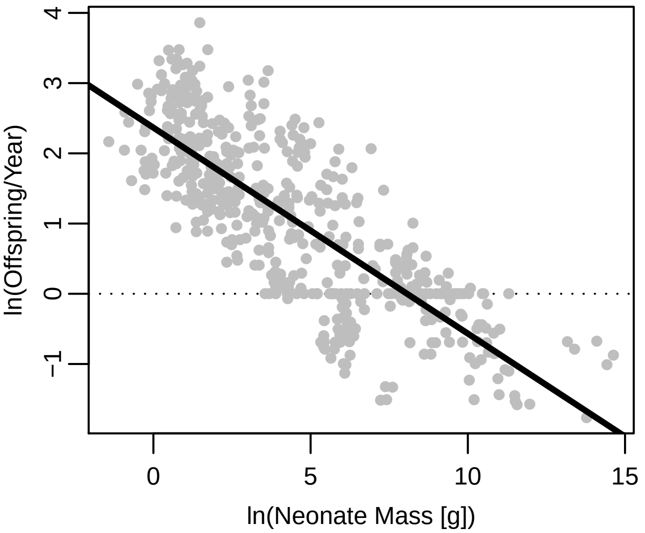

Figure 0.1: Trade-off between natural log of offspring number and natural log of offspring size in 532 species of mammals corrected for phylogenetic relationships. As offspring size increases, offspring number decreases (slope = -0.30). Modified after Charnov and Ernest (2006).

Koufopanou 1985; Reznick 1985; Pease and Bull 1988; Stearns 1989; Reznick et al. 2000; Roff 2000; Roff 2002). Many cases have been documented where expected trade-offs are not found experimentally (Biere 1995; Czesak and Fox 2003), and occasionally correlations between traits thought to be involved in a trade-off are positive rather than negative (Gebhardt and Stearns 1988; Spitze et al. 1991; Genoud and Perrin 1994; Yampolsky and Ebert 1994; Jordon and Snell 2002; Messina and Fry 2003; Ernande et al. 2004; Vorburger 2005). Furthermore, observed trade-off patterns within populations often vary depending upon the environment in which they are measured (Bell and Koufopanou 1985; Gebhardt and Stearns 1988; Kaitala 1991; Stearns et al. 1991; Leroi et al. 1994; Czesak and Fox 2003; Messina and Fry 2003; Blanckenhorn and Heyland 2004; Ernande et al. 2004; Sgrò and Hoffmann 2004). Thus, empirical studies reveal considerable variation in the correlation between traits thought to be involved in resource based trade-offs. However, the causes of this variation and their consequences for life history evolution remain poorly understood.

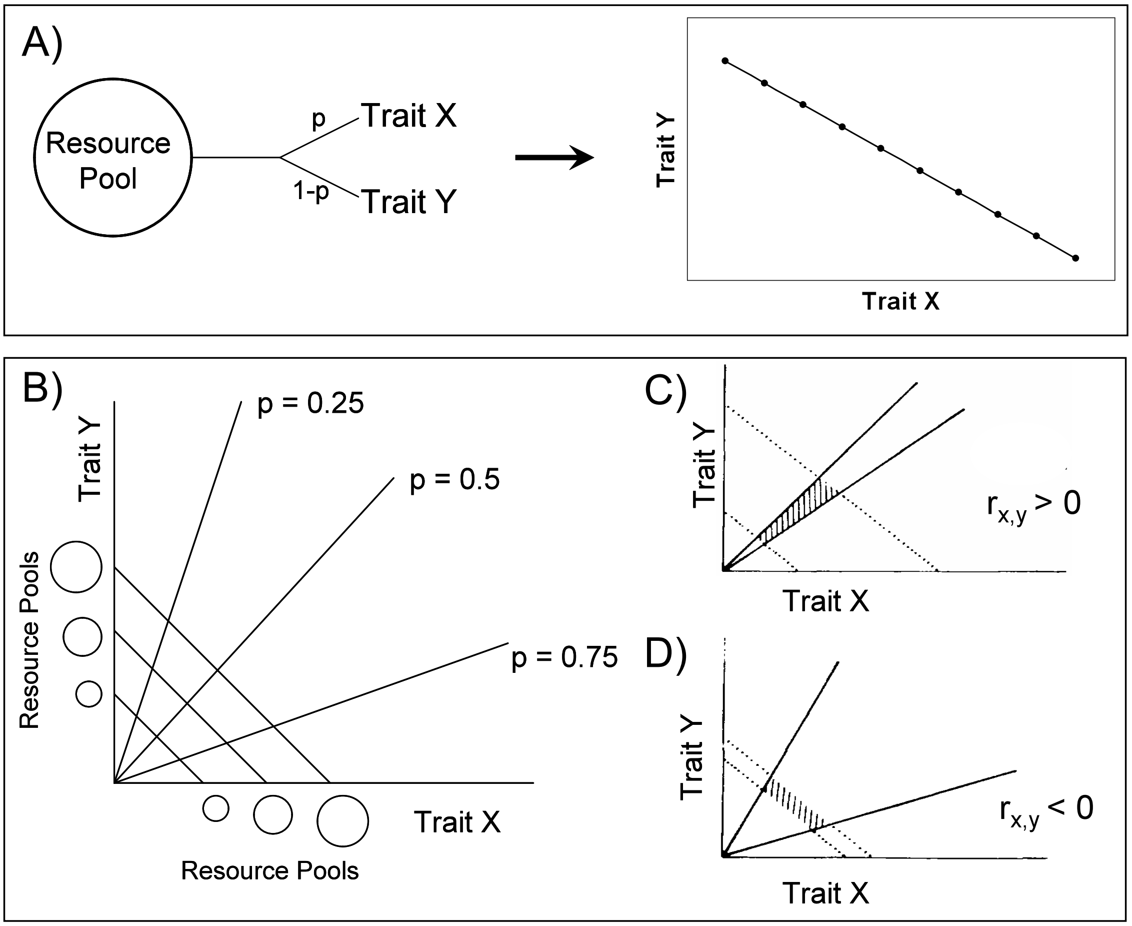

Figure 0.2: A) Simple Y model with two traits, X and Y pulling resources from a single fixed resource pool. A proportion, p is allocated to trait X and $1-p$ is allocated to Y. This leads to a negative correlation between X and Y. B–D) Modified from van Noordwijk and de Jong (1986). B) The range of trait values possible for different resource pools and for different values of p. C) The case where variation in resource pool (acquisition) is large and variation in p (allocation) is small, generating a positive correlation. D) The case where variation in resource pool (acquisition) is small and variation in p (allocation) is large, generating a negative correlation.

One explanation for the variation in observed trade-off patterns is the Y model, developed by van Noordwijk and de Jong (1986). These authors showed that the expression of trade-offs depends both on variation in the acquisition of resources and in the subsequent allocation of those resources. Their model consists of two traits both drawing from a common resource pool. For a fixed acquisition (i.e., resource pool size), variation in allocation leads to a negative correlation between traits (Figure 0.2A). However, if acquisition varies more than allocation, some individuals will have more resources to allocate to all traits than others, and a positive correlation is present between the traits (Figure 0.2B–D). Specifically, the Y model predicts that the sign of the correlation depends on the relative variation in acquisition and the relative variation in allocation. It follows that any genetic and or phenotypic sources of variation in the acquisition of resources and subsequent allocation of those resources will influence the expression of trade-offs in natural populations.

Given that variation in acquisition relative to variation in allocation is predicted govern trade-off patterns, to understand how and why trade-offs vary in natural populations, we must study the sources of variation. Substantial genetic variation may exist for the acquisition and/or allocation of resources. These traits may also be phenotypically plastic in the sense that the phenotypic value of a genotype changes in response to the environment. Phenotypic variation is not exclusive of genetic variation; genetic variation for a plastic response can and, indeed, must exist for phenotypic plasticity to evolve. This variation in a plastic response among genotypes is referred to as a genotype by environment interaction (Pigliucci 2001). These three sources of variation—genotype, environment, and genotype by environment interaction—are critically important in determining trade-off patterns. In my dissertation, I use a model system to investigate these potential sources of variation in acquisition and allocation: the life history trade-off between flight capability and reproduction in wing dimorphic insects.

Wing dimorphic insects have been studied extensively and have emerged as a model system for the study of trade-offs (Harrison 1980; Roff 1986a; Dingle 1996; Zera and Denno

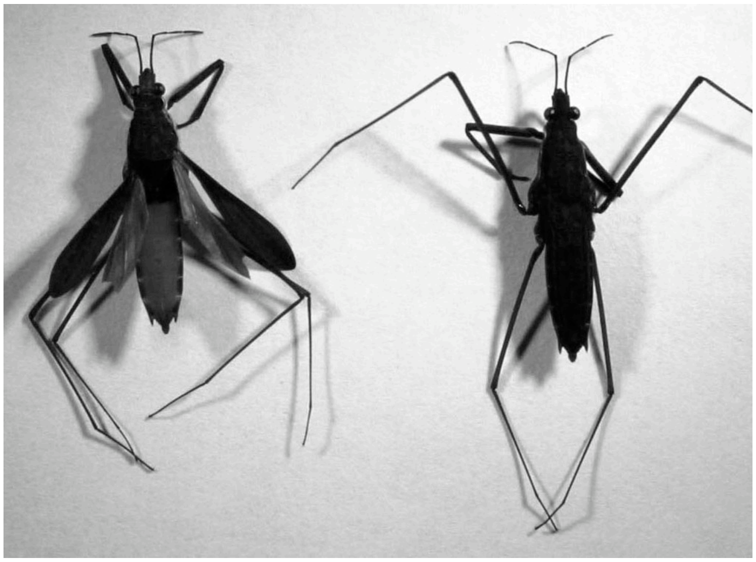

Figure 0.3: An example of a wing dimorphic species: the water strider, *Aquarius remigis.*

1997). Two discrete morphs exist, a macropterous, flight capable morph with fully developed functional flight apparatus and a flightless morph that is either micropterous, with greatly reduced wings or apterous, completely lacking wings (Figure 0.3). The ability to fly confers the fitness benefit of long-range dispersal. Both comparative and theoretical studies have found that in habitats that are ephemeral, macropters will be favored due to their ability to colonize new habitats and escape deteriorating ones (Roff 1974a, b, 1994b; Vespäläinen 1978; Harrison 1980; Dingle 1996). Given the advantage of flight capability, the persistence of short winged individuals in the population implies that some cost is associated with the ability to fly. Flightless morphs across species show an increased fecundity in comparison with flight capable morphs (Harrison 1980; Roff 1984, 1986a; Dingle 1996; Zera and Denno 1997). Even in the absence of flight, making and maintaining the large flight muscles incurs a significant energetic cost (Harrison 1980; Roff 1984, 1986a; Dingle 1996; Zera and Denno 1997; Zera et al. 1997; Crnokrak and Roff 2002). Many studies on a diversity of wing dimorphic species have demonstrated a trade-off between flight capability and reproduction (for reviews see Harrison 1980; Roff 1986a; Dingle 1996; Zera and Denno 1997), and these studies provide a strong argument that these two traits are in a functional trade-off and compete for resources. This conclusion has been confirmed by physiological studies demonstrating differences in the allocation of available nutrients in flight capable compared to flightless morphs (Zera et al. 1994; Zera and Denno 1997; Zera and Brink 2000; Zera 2005; Zhao and Zera 2006).

An important source of variation in allocation is phenotypic plasticity, the response of allocation to environmental cues. In wing dimorphic insects, allocation occurs at two levels, both of which may be phenotypically plastic and respond to environmental cues (e.g., Vespäläinen 1978; Denno et al. 1985; Roff 1986; Zera and Tiebel 1988; Kaitala 1991; Zera and Tiebel 1991; Roff and Shannon 1993; Shimizu and Masaki 1993; Fairbairn and Yadlowski 1997; Harada et al. 1997; Sasaki et al. 2002; Braendle et al. 2006). First, nymphs develop into either flight-capable or flightless morphs. Second, once they are adults and have a fixed wing morph, winged individuals allocate some proportion of their resources to flight capability and some proportion to reproduction. The conditions under which allocation to flight capability versus reproduction should shift and when one pattern of plasticity will be favored over another are unknown.

In chapter one, I characterize the evolutionary potential of phenotypic plasticity in wing morphology in response to environmental variation in temperature in the wing dimorphic water strider, *Aquarius remigis* (Figure 0.3). Almost all wing dimorphic water strider species show phenotypic plasticity in wing morph induction (e.g., Vespäläinen 1978; Calabrese 1979; Harrison 1980; Andersen 1982; Zera et al. 1983; Fairbairn 1985; Zera 1985; Spence 1989; Zera and Tiebel 1991; Dingle 1996; Harada et al. 1997; Pfenning and Poethke 2006). Most of these studies presume that patterns of phenotypic plasticity in wing dimorphism are adaptive. However, no studies have investigated the quantitative genetics of plasticity in wing dimorphism in any species and therefore we have no information about how or if the pattern of phenotypic plasticity can evolve in response to selection. My study tests the assumptions that wing dimorphism and the response of wing dimorphism to changes in the environment have the potential to evolve in *A. remigis* by estimating the genetic architecture of wing dimorphism in two temperature environments.

In chapter two, I consider phenotypic plasticity in both wing morph induction and in the proportional allocation of resources to flight capability and reproduction. I use a quantitative genetic simulation modeling approach to predict how phenotypic plasticity in resource allocation will evolve in response to spatiotemporal variation in resource levels in wing dimorphic insects. How optimal allocation strategies change in response to resource availability in any given ecological situation is a neglected, yet potentially very important area of biology. In nature, resource availability varies spatially and temporally, both within and across generations, leading to variation in the amount of energy available to individuals. The optimal allocation strategy may change depending upon the amount of resources available. If so, selection should favor the evolution of phenotypically plastic allocation strategies that can be altered in response to variation in environmental resource levels. Many examples of shifts in resource allocation in response to variation in resource levels in natural populations have been described (e.g., Gebhardt and Stearns 1988; Kaitala 1991; Chippendale et al. 1993; Ellers and van Alphen 1997; Blanckenhorn 1998; Tessier et al. 2000; Jordan and Snell 2002; Czesak and Fox 2003; Messina and Fry 2003; Ernande et al. 2004; Bashey 2006; Ruf et al. 2006), however, theoretical models predicting how the phenotypic plasticity in allocation should evolve are lacking. In this chapter, I bridge this gap.

In chapters three, four and five, I utilize the wing dimorphic sand cricket, *Gryllus firmus* (Orthoptera, Gryllidae) to study both genetic and phenotypic variation in acquisition and allocation. Much of what is known about wing dimorphisms comes from extensive work done on *Gryllus firmus*. Gryllus firmus ranges from costal Texas through Florida and up to Connecticut, inhabiting sandy and early successional sites (Alexander 1968; Figure 0.4). The trade-off between flight capability and fecundity in *G. firmus* has been well studied from both a quantitative genetic (Table 0.1a) and a physiological perspective (Table 0.1b). Quantifying the sources of

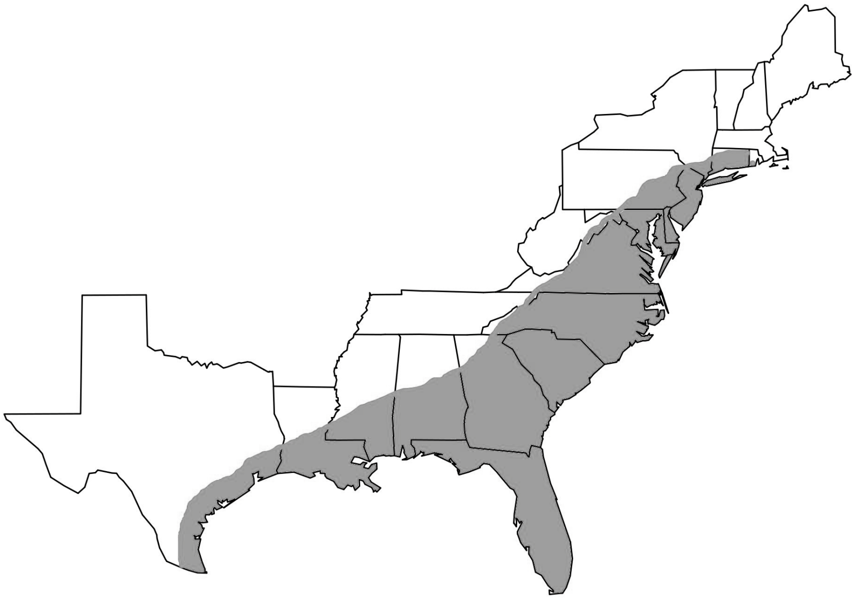

Figure 0.4: Natural range of the Sand Field Cricket (*Gryllus firmus*)

variation in acquisition and allocation requires an integration of quantitative genetics and the physiological mechanisms that determine acquisition and allocation patterns, making *G. firmus* an ideal study system to empirically address variation in acquisition and allocation.

A serious challenge to any study of acquisition and allocation is reliably measuring these two variables, which requires quantifying both an individual's total resource pool and the proportion of those resources allocated to various traits. In chapter three, I estimate energy acquisition and energy allocation in a population of female *G. firmus*, by measuring both the biochemical composition of different body tissues and the whole organism resting metabolic rate for individuals reared on two food levels. From these measurements, I formulate a predictive model showing that the energy allocated to reproduction and flight capability can be estimated from the masses of ovaries and flight muscles. The ability to estimate relative acquisition and allocation from simple mass measurements significantly simplified the experiments described in chapters four and five where detailed biochemical measurements were not feasible.

Table 0.1: Examples of a) quantitative genetic studies and b) physiological studies utilizing *Gryllus firmus*.

a) *Quantitative Genetic Studies*	
Wing morphology is polygenic with a high heritability	Roff 1986
A significant negative genetic correlation has been identified between DLM mass and fecundity	Roff and Gelinas 2003
A significant negative genetic correlation has been identified between the state of DLM (degree of histolysis) and fecundity	Stirling et al. 1999; Roff 1994
Significant genetic correlations have been found between the proportion macropterous and fecundity, propensity to migrate and degree of histolysis	Fairbairn and Roff 1990; Roff 1994
Quantitative genetic analysis of phenotypic plasticity to food ration indicates a high genetic correlation between environments for both fecundity and DLM mass	Roff and Gelinas 2003
b) *Physiological Studies*	
Isolated fully developed DLM have a significantly greater respiration rate than underdeveloped or partially histolyzed DLM	Zera et al. 1997
Total respiration rate is greater for macropterous females relative to micropterous females	Crnokrak and Roff 2002
Macropterous females have greater biosynthesis of lipids and higher levels of triglycerides (primary flight fuel) than micropterous females	Zera 2005; Stirling et al. 2001; Zera and Larsen 2001
The two wing morphs do not differ in the amount of nutrients (lipid, nitrogen, carbohydrate) assimilated from their diet	Zera and Brink 2000

The Y model predicts that genetic and phenotypic variation in the acquisition of resources and the subsequent allocation of those resources to different traits can profoundly affect the correlation between traits involved in a functional trade-off (van Noordwijk and de Jong 1986). In chapter four, I evaluate the major sources of variation in acquisition and allocation—genotype, environment, and genotype by environment interaction—by performing a large-scale quantitative genetic breeding experiment, in which half-sibling *G. firmus* families are split over three resource levels. Using this design, I am able to estimate genetic and phenotypic variance components in multiple environments and evaluate the evolutionary genetics of phenotypic plasticity in acquisition and allocation.

In the final chapter, I utilize the data set from chapter four as a case study to test several outstanding assumptions and predictions of the Y model. While this model has been very influential in the field of life history evolution (Reznick et al. 2000), it has rarely been tested. Here, I am able to utilize the model developed in chapter three to estimate acquisition and allocation in units of energy in order to directly test the Y model. In addition, I propose principal components analysis as a new method to extract the underlying factors—namely acquisition and allocation—determining trade-offs.

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Chapter 1

The evolutionary genetics of wing dimorphism in a water strider

1.1 Introduction

Movement away from a place of origin or from one habitat to another is an innate and necessary attribute of all organisms (Chepko-Sade and Halpin 1987; Stenseth and Lidicker 1994; Dingle 1996; Clobert et al. 2001). Often such movements are termed “dispersal” when viewed at the population level, but “migration” when referring to the behavior of individuals, particularly if this is active and appetitive (Danthanarayana 1986; Taylor 1986; Dingle 1996; Dingle and Drake 2007), a policy we shall adopt in this paper. Most simply put, the migratory behavior of individuals results in their dispersal among habitat patches or across the landscape. Migration has obvious benefits at the population and individual levels: it permits colonization of new habitats and escape from deteriorating habitats, reduces inbreeding and increases effective population size, facilitates the spread of advantageous alleles, and generally reduces the risk of lineage extinction. However, at the individual level, migration also carries significant costs:

migrants often face higher risks of predation and may fail to find a suitable breeding habitat. Further, the energy required for migration must be diverted from other life history components, often requiring a delay in the onset of reproduction or a reduction in reproductive output (Roff 1984, 1986; Roff and Fairbairn 1991, 2007; Dingle 1996). Given these costs and benefits, it is not surprising that migratory tendency varies greatly within and among species (Dingle 1972, 1996; Roff 1990, 1994a, b; Chepko-Sade and Halpin 1987; Wagner and Liebherr 1992; Stenseth and Lidicker 1994; Andersen 1997; Clobert et al. 2001).

Although variation in migratory tendency can be predicted (*op. cit.*), testing hypotheses concerning the adaptive significance and evolutionary dynamics of this variation in natural systems poses significant challenges, not the least of which is measuring migratory capability. Insects have proven to be excellent model organisms for such studies because migratory capability is often overtly expressed in the form of wing polymorphisms: migratory morphs (macropters) have fully-formed wings and are generally capable of flight at some phase of their adult life, while non-migratory morphs have reduced wings (brachypters) or no wings at all (apters) and are incapable of flight (Dingle 1972, 1985, 1996; Harrison 1980; Roff 1986, 1990; Taylor 1986; Roff and Fairbairn 1991, 2007; Wagner and Liebherr 1992; Zera and Denno 1997; Andersen 1997). Although wing reduction or loss may seem anomalous in a Class of organisms characterized by the evolution of flight, we now understand that it occurs because of fundamental trade-offs between migratory and reproductive capabilities. In particular, the muscles and fuels required for flight use resources that could be devoted to reproduction (Zera and Denno 1997; Zera and Brink 2000; Zera 2005), and as a result, macropters tend to have reduced reproductive output relative to sedentary morphs (Roff 1986; Denno et al. 1989; Fairbairn and Roff 1991, 1997; Zera and Denno 1997). Most commonly, the trade-off between migratory capability and reproduction is manifest primarily as reduced fecundity of macropterous females, but increased development time and reduced male mating success also occur in some species. As a consequence of these trade-offs, wing reduction has evolved repeatedly in clades of insects where flight is not necessary for normal foraging and reproductive activity. In such clades, the occurrence of wing reduction correlates strongly with habitat persistence: macroptery predominates in environments where habitat persistence is low, while wing reduction is favored in permanent, stable habitats because of the overall higher reproductive capacity of the flightless morph (Southwood 1962; Harrison 1980; Roff 1974a, b, 1986, 1994a; Taylor 1986; Spence 1989; Roff and Fairbairn 1991, 2007; Dingle 1996; Andersen 1997; Zera and Denno 1997).

The evolutionary ecology of wing reduction and flight polymorphism has been particularly well-studied in temperate water striders, a clade of surface-dwelling, semi-aquatic bugs (Hemiptera-Heteroptera, Gerridae, Gerrinae; e.g., Andersen 1973, 1982, 1993, 1997, 2000; Calabrese 1974, 1979; Vepsäläinen 1978; Vepsäläinen and Patama 1983; Zera 1984, 1985; Fairbairn 1986, 1988; Fairbairn and Desranleau 1987; Spence 1989; Fairbairn and Butler 1990; Kaitala 1991; Kaitala and Dingle 1992; Harada et al. 1997; Ahlroth et al. 1999; Pfenning and Poethke 2006). The disadvantage of the macropterous morph in terms of delayed reproductive maturity, reduced fecundity or reduced mating success has been demonstrated in many species (Vepsäläinen 1978; Andersen 1982; Zera 1984, 1985; Fairbairn and Desranleau 1987; Fairbairn 1988; Spence 1989; Roff and Fairbairn 1991; Harada 1992; Kaitala and Dingle 1993; Fairbairn and Preziosi 1996). There is also strong support for the relationship between habitat persistence and wing reduction, with primarily macropterous species being found in smaller, discontinuous, temporary habitats such as ponds and sloughs, and primarily apterous species in more continuous, permanent habitats such as large lakes, streams and small rivers (Vepsäläinen 1978; Calabrese 1979; Spence and Scudder 1980; Andersen 1982, 1993, 1997, 2000; Spence 1989; Fairbairn and Butler 1990). The conclusion from these studies is that in the Gerrinae, as in other insect groups, wing dimorphisms are maintained by the fundamental trade-off between migratory capability and reproduction, and that the balance between the flight-capable and flight-incapable morphs is largely determined by habitat persistence.

While these studies have yielded much information on the evolutionary ecology of wing dimorphism in water striders, they carry the implicit assumption that wing morphology has the genetic architecture to evolve at the local and landscape scale and is not constrained by low heritability or genetic correlations with other traits, an assumption that has not been tested. Although the heritability of wing morphology has not been estimated for any water strider species, several studies have demonstrated a genetic basis for wing dimorphism in this clade, the data being most consistent with a polygenic mechanism of morph determination rather than a single locus (Vepsäläinen 1978; Zera et al. 1983; Roff 1986; Spence 1989). However, Fairbairn (1988) found no evidence for a genetic effect on wing morphology in an eastern population of *A. remigis*. Thus it is unclear whether the varied frequencies of wing morphology among populations and species of water striders reflects genetic adaptation or purely phenotypic responses to environmental cues.

Nearly all dimorphic water strider species show phenotypic plasticity in proportion macropterous most often expressed as a variable proportion of the flightless morph in the summer, non-diapausing generation (Vepsalainen 1978; Calabrese 1979; Harrison 1980; Andersen 1982; Fairbairn 1985; Zera 1985; Spence 1989; Dingle 1996; Pfenning and Poethke 2006). In addition to this general pattern, changes in proportion macropterous have been found in response to photoperiod (Vepsäläinen 1978; Zera et al. 1983; Spence 1989; Zera and Tiebel 1991), density (Harada et al. 1997) and temperature (Fairbairn 1985; Pfenning and Poethke 2006). A majority of this work presumes that patterns of phenotypic plasticity (reaction norms) in wing dimorphism are adaptive, particularly to variation in season length (e.g., Vepsäläinen 1978; Zera et al. 1983; Spence 1989; Zera and Teibel 1991; Pfenning and Poethke 2006). However, no studies have investigated the quantitative genetics of plasticity in wing dimorphism in any species and therefore we have no information about how or if the pattern of phenotypic plasticity can evolve in response to selection.

In the present study, we aim to determine the quantitative genetic architecture of wing dimorphism in a Californian population of the stream-dwelling water strider *Aquarius remigis* Say. *Aquarius remigis* is one of the most common and abundant Palearctic water strider species and occupies the surfaces of streams and small rivers throughout much of temperate North America (Scudder 1971; Calabrese 1979; Polhemus and Chapman 1979; Andersen 1990; Preziosi and Fairbairn 1992; Gallant and Fairbairn 1996). *A. remigis* are primarily apterous through most of their range, with macropters occurring at a frequency of less than 1% (Scudder 1971; Calabrese 1974, 1979; Fairbairn 1985; Andersen 1990; D.J. Fairbairn, *unpublished data*). In contrast, Californian populations of *Aquarius remigis* have a high incidence of macroptery, comprising up to 100% of adults in some populations (Calabrese 1974; Polhemus and Chapman 1979; Andersen 1990; Kaitala and Dingle 1992; Fairbairn and Preziosi 1996) leading to the hypothesis that selection has favored a higher incidence of macroptery in Californian populations (Kaitala and Dingle 1992; Fairbairn and King 2009). In addition, while the incidence of wing dimorphism most commonly responds to photoperiod in water striders, previous studies of Californian *A. remigis* have demonstrated an independent effect of temperature and have hypothesized that this response is adaptive (Fairbairn and King 2009). Our study tests the assumptions that wing dimorphism and the response of wing dimorphism to changes in the environment have the potential to evolve in Californian populations of *A. remigis* by estimating the genetic architecture of wing dimorphism in multiple temperature environments.

For this study, we use a half-sibling design, split across two rearing temperatures so that the influence of genes, environment (temperature), and their interaction can be determined. We are able to estimate the additive genetic variance and heritability of wing morphology and splitting each dam family across two rearing temperatures in this experiment also enables us to estimate the genetic correlations across environments and to test for a significant genotype by environment interaction (“G × E”). Significant additive genetic variance and heritability for wing morphology would indicate that wing morph frequency has the potential to respond to selection, while a significant G × E would suggest a similar potential for plasticity of wing morph expression in response to temperature.

1.2 Methods

The source population for our half-sibling laboratory breeding experiment consisted of 61 male and 64 female reproductively mature *A. remigis* captured on March 13, 2005 from a permanent stream in Rattlesnake Canyon, Santa Barbara, California at an altitude of 256 m. This is a low altitude coastal stream in the Mediterranean climate zone.

The field-caught adults were maintained in laboratory stream tanks (135 cm × 27 cm) at room temperature (mean 22.8 ° C, range 17 ° C–27 ° C) and a 14h photophase equivalent to day length in mid-May or late July in their native site (N 34.4°). Eggs were collected semi-weekly and the F1 nymphs reared under the conditions described above. All striders were provided daily with food (frozen *Drosophila melanogaster* and *Acheta domestica*) beyond the amounts required to maintain full fecundity and maximum survival to adult eclosion (Blanckenhorn et al. 1995). The first lab-reared generation (F1) served as parents for the half-sibling experiment described below.

Parents for the half-sibling breeding experiment were chosen randomly from the F1 generation and individually numbered with Testors' Enamel. The females (dams) were placed in plastic cages (34 × 20 × 10 cm) in the 25 ° C growth chamber. Each male was assigned at random to three dams, and was rotated among the three dam cages, changing cages every two days. All cages received scored Styrofoam pieces as oviposition sites and resting spots. Eggs were collected twice a week from each dam and apportioned equally among four identical plastic cages (34 × 20 × 10 cm), two in a 20 ° C growth chamber and two in a 25 ° C growth chamber, until each cage contained approximately 30 eggs. The lower temperature approximates the average daily mean temperature in the coastal region of Santa Barbara during the summer (July–September; http://www.santabarbara.com/community.weather). The higher temperature approximates the mean daily high temperature during mid-summer (August), and much higher temperatures (32 ° C or higher) have been recorded in all months (*op. cit.*). Thus, the temperatures used for our half-sib experiment are well within the range of temperatures normally experienced by individuals in this population. Within each growth chamber, families were placed in a randomized block design, blocked with respect to shelf height and distance from the chamber door, to control for possible light and temperature gradients within the chamber. All striders were provided with food (frozen *Drosophila melanogaster* and *Acheta domestica*) daily, as above. Cages were checked daily and all eclosed adults were removed and scored for wing morphology. The final data set consisted of 36 sires, 104 dams, and 2,200 offspring.

1.2.1 Statistical Analysis

To test for the effects of family, temperature and their interaction, we performed a nested, mixed model ANOVA with sire and dam as random effects and temperature as a fixed effect (Fry 1992; Shaw and Fry *unpublished data*). All effects were estimated using the traditional least squares method and the significance of random effects were then calculated according to the methods of Zar (1996). However, our response variable, wing morphology, is a dichotomous variable and violates the assumption of normality inherent in parametric ANOVA. We therefore performed a randomization with 10,000 iterations to confirm the results of the ANOVA and we report the probabilities from both analyses.

Previous studies indicate that wing morphology in water striders is a polygenic, dichotomous trait (*op. cit.*), and we therefore used a threshold model to estimate its heritability (Roff 1996, 1997). The threshold model assumes that the discrete trait, in this case aptery/macroptery, is the result of an underlying, continuously distributed trait termed the liability. Individuals with liabilities below a certain threshold level develop into macropters and those with liabilities above that level develop into apters. Using this model, one actually estimates the heritability of the liability determining wing morphology (Roff 1997), but for brevity we refer to this simply as the heritability of wing morphology. We calculated the heritabilities using variance components estimated using restricted maximum likelihood analysis followed by a delete-one jackknife, as the jackknife has been shown to perform well for estimating both heritability (Knapp et al. 1989; Simons and Roff 1994) and genetic correlations (Roff and Preziosi 1994). The jackknife also has the advantage of performing well with unbalanced data while other methods do not (Simons and Roff 1994). In the delete-one jackknife, one sire family is deleted and a "pseudovalue" of heritability is calculated. This process is repeated, eliminating each sire family once. The mean and standard error of the full set of pseudovalues estimate the heritability and its standard error (Roff 2006). To account for possible differences between temperatures and/or sexes, we estimated heritabilities separately for half-sib (sire) families and full-sib (dam) families for each sex and each environment and then tested for differences among our separate estimates of heritability using a two-way ANOVA based on the pseudovalues (because the distribution of the pseudovalues is the same as that of the heritabilities this test is a test for variation in heritability). All analyses for the half-sib experiment were done using S-PLUS$^{\copyright}$ Statistical Software (Version 6; Insightful Corporation).

1.3 Results

The ANOVA testing for effects of sire, dam and temperature was highly significant (Table 1.1). The main effect of sire indicates the presence of highly significant additive genetic variance for wing morphology, while the main effect of temperature indicates that wing morphology shows phenotypic plasticity in response to temperature. The significant sire by temperature interaction indicates significant genetic variation for this phenotypic plasticity.

Table 1.1: Results of mixed model analysis of variance of wing morphology as a function of sire, dam and temperature. P_{rand} refers to the probabilities generated from 10,000 randomizations (see text).

Source	**d.f.**	**MS**	***F***	***P***	$\boldsymbol{P}_{\text{rand}}$
Sire	35	0.58	2.84	<0.001	0.0001
Dam(sire)	68	0.21	1.91	<0.001	0.0002
Treatment	1	1.63	7.30	0.011	0.0098
Sire × Treatment	35	0.22	1.83	0.019	0.0114
Dam(sire) × Treatment	60	7.32	1.14	0.23	0.2287
Residuals	2000	0.11			

The heritability estimates did not differ based on temperature (Sire: $F_{1,140}$ = 1.30, *P* = 0.26; Dam: $F_{1,140}$ = 0.19, *P* = 0.66), sex (Sire: $F_{1,140}$ = 0.28, *P* = 0.60; Dam: $F_{1,140}$ = 0.57, *P* = 0.45) or their interaction (Sire: $F_{1,140}$ = 1.11, *P* = 0.29; Dam: *F1,140* = 0.64, *P* = 0.42). We therefore pooled the pseudovalues and estimated overall heritabilities and standard errors for half-sib (sire) and full-sib (dam) families. For the estimate of the standard error, we used an N of 36 (the number of sire families) and not the number of pseudovalues where each family is represented four times (two environments and two sexes). These estimates are high (sire: 1.32, SE = 0.92; dam: 0.83, SE = 0.70) though they have very wide confidence intervals.

The heritability estimate based on sire families is an estimate of heritability in the narrow sense and includes only additive genetic variance, while the estimate using dam families also includes a portion of any non-additive effects present. To test for significant differences between the sire and dam estimates and hence determine if there are significant non-additive effects, we performed a delete-one jackknife (Roff 2007). In this method, each sire family is deleted and a sire pseudovalue and a dam pseudovalue are calculated. This results in a set of paired samples (sire and dam estimates) and a one-tailed paired *t*-test can be used to determine if the dam estimate significantly exceeds the sire estimate (Roff 2007). We found no difference between the sire and dam estimates of heritability (t_{143} = 0.668, *P* = 0.75). Further, contrary to expectations, the dam estimate was less than the sire estimate. Thus, even though the power of the comparison between dam and sire estimates can be low (Roff 2007), we are confident that non-additive effects on wing morphology in *A. remigis* are minimal. In the absence of non-additive effects, the most accurate estimate of heritability is the mean of the sire and dam estimates, called the genotypic estimate of heritability, which has a much smaller standard error (Roff 2007). Our estimate of genotypic heritability is very high (h^2 = 1.08, SE = 0.33) and significant (95% CI = 1.75–0.39).

We estimated the genetic correlation between males and females separately for each temperature using covariance and variance components estimated using restricted maximum likelihood analysis. We again followed this analysis with a delete-one jackknife and used the pseudovalues to test for differences between the sire and dam estimates of the genetic correlation. Finding no differences for either temperature (20 °C: $t_{35} = -0.34$, $P = 0.74$; 25 °C: $t_{35} = -0.024$, P = 0.98), we estimated the genotypic genetic correlation. The genotypic estimates of the genetic correlation between males and females are very high (20 °C: $r_g = 0.93$, SE = 0.12; 25 °C: $r_g = 0.82$, SE = 0.12), indicating the genetic determinants of wing morphology are very similar in the two sexes.

The genetic parameters indicate strong genetic effects on wing morphology in *A. remigis* but comparison of the two temperature treatments indicates significant phenotypic plasticity as well. The proportion macropterous was significantly affected by temperature ($F_{1,35} = 7.3$, $P = 0.011$) and was lower at 25 °C than at 20 °C (25 °C: mean = 0.11, SE = 0.0003 ; 20 °C: mean = 0.20, SE = 0.0003; Figure 1.1). We examined the quantitative genetic basis of this phenotypic plasticity using the approach of Via and Lande (1985) to estimate the genetic correlation between the two environments. We estimated the covariance and variance components using restricted maximum likelihood analysis and used a delete-one jackknife to test for differences among the sire and dam estimates. Finding no differences between sire and dam estimates, ($t_{35} = 0.28$, P = 0.78), we used the genotypic estimate of the genetic correlation between environments. This estimate is significant but less than 1.0 ($r_g = 0.63$, SE = 0.23), indicating

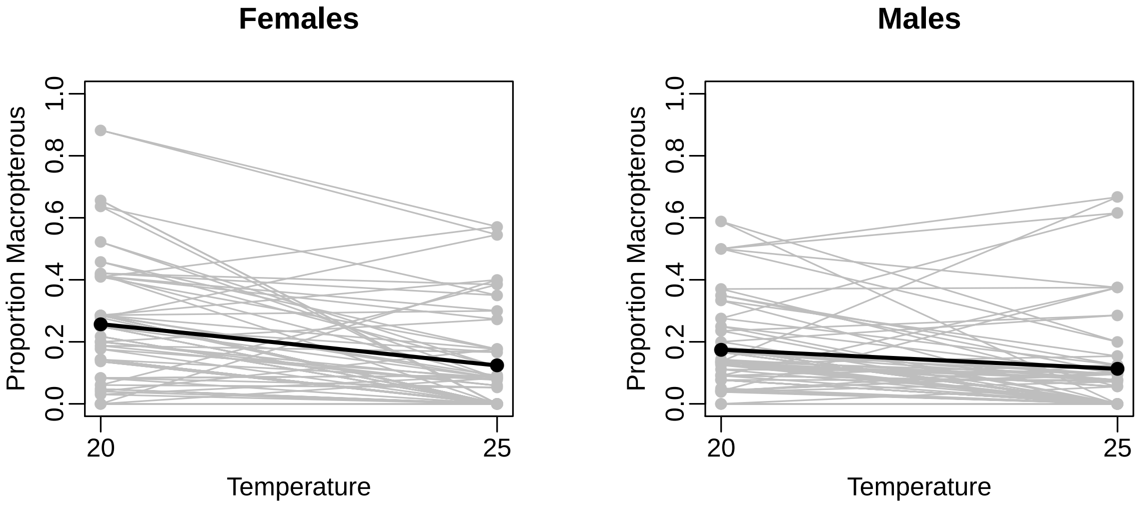

Figure 1.1: Reaction norms showing the response of the proportion of macropterous individuals in response to temperature for females and males. Grey lines show responses of individual sire families while the black line shows the average response in the population.

similarity among families in their response to temperature, but also genetic variance in this response. An estimate of the genetic correlation between temperatures that is different than one indicates that families are not perfectly correlated between environments and therefore that families differ in their response to temperature. The significant sire by temperature interaction in the overall ANOVA (Table 1.1) confirms that there is indeed significant genetic variation in the response of wing morphology to temperature. This is evident in the varied reaction norms displayed by sire families (Figure 1.1).

1.4 Discussion

Our results have demonstrated that wing morphology is highly heritable in this population of *A. remigis*, indicating that wing morphology can respond rapidly to selection. Further, we were able to demonstrate very high levels of additive genetic variation and high heritability under two different rearing temperatures, indicating that the potential for wing morphology to respond to selection on a local scale is unlikely to be masked by developmental responses to local temperature regimes. The high heritability of wing morphology in *A. remigis*, the first estimated for any water strider species, is consistent with estimates for other wing dimorphic insects, which are typically greater than 0.50 (reviewed in Roff and Fairbairn 1991, 2007; Dingle 1996). This validates the use of water striders as a model system to study the evolution of wing dimorphism and adds support to the many earlier studies that have constructed adaptive arguments based on the implicit assumption that wing morph frequency responds readily to selection on local scales (e.g., Vepsäläinen 1978; Andersen 1982, 2000; Fairbairn 1988; Kaitala and Dingle 1992; Ahlroth et al. 1999; Pfenning and Poethke 2006).

In particular, Californian populations of *A. remigis* have long been noted for their high frequency of macropters relative to populations in other regions of North America (Calabrese 1974; Polhemus and Chapman 1979; Kaitala and Dingle 1992). The high heritability of wing morphology in our Californian population of *A. remigis* indicates that the frequency of macropters in these populations can and has responded readily to selection. Fairbairn and King (2009) found that Californian populations of *A. remigis* differ genetically in the proportion of macropterous individuals. Our finding of a high heritability of wing morphology, combined with the findings of Fairbairn and King (2009) support the hypothesis that the high incidence of macroptery in Californian populations reflects genetic adaptation of these populations to the unique environmental characteristics of that region.

In insects with polygenic wing morph determination, wing morph induction is typically determined by an interaction between the genetic predisposition to produce wings and environmental variables during key developmental periods (Dingle 1972, 1996; Roff 1986, 1996, 1997; Roff and Fairbairn 1991, 2007; Zera and Denno, 1997). In water striders, previous studies have shown that wing morphology responds to photoperiod (Vespäläinen 1978; Zera et al. 1983; Spence 1989; Zera and Tiebel 1991), density (Harada et al. 1997) and temperature (Fairbairn 1985, 1988; Pfenning and Poethke 2006). The majority of studies have emphasized responses to photoperiod (e.g., Vespäläinen 1978; Andersen 1982; Zera et al. 1983; Spence 1989; Zera and Teibel 1991; Harada and Numata 1993), and where temperature has been studied, the general assumption has been that it acts primarily through its interaction with photoperiod (Andersen 1982; Fairbairn 1985, 1988; Pfenning and Poethke 2006). However, Fairbairn and King (2009) demonstrated a direct effect of temperature that is independent of variation in photoperiod in several Californian populations of *A. remigis*, a finding that was reproduced in this study. The response of wing dimorphism to temperature will be particularly important in landscapes with significant altitudinal gradients. In California, the climate at any given latitude varies dramatically with both altitude and distance from the coast (Felton 1965; Tritenbach 1999), and hence photoperiod alone is likely to be a relatively poor predictor of season length or habitat suitability. In such a landscape, selection should favor incorporation of cues other than or in addition to photoperiod to synchronize life history transitions with seasonal habitat suitability. Californian populations of *Aquarius remigis* may use temperature as such a cue. However, for the response of wing dimorphism to temperature to evolve in *A. remigis*, it must have the genetic architecture to do so.

In water striders, as in other wing dimorphic clades, the responses of wing morphology to environmental cues are generally assumed to be adaptive (Fairbairn 1985; Andersen 1982; Dingle 1986; Pfenning and Poethke 2006). However, until now, the quantitative genetics of that plasticity had not been examined. We found a significant genetic correlation between temperatures, indicating that on average, genotypes that tend to produce high levels of macroptery at one temperature also do so at the other temperature. However, the relatively modest magnitude of the between-environment correlation leaves room for considerable genetic variation in phenotypic plasticity and the presence of such variation was confirmed by the significant sire by temperature interaction. This study is the first to demonstrate a significant genotype by environment interaction for wing dimorphism. This result indicates that the response of wing dimorphism to temperature in *A. remigis* can evolve and is consistent with the hypothesis that

Californian populations use local temperature, at least in part, as a cue for of season length and/or habitat suitability.

A significant amount of what is known about the evolution of migratory ability has come from studies utilizing water striders as a model system. This study is a first step toward a greater understanding of the quantitative genetics of wing dimorphism in this system. It provides critical information about how evolution will proceed in this system and lends support to previous studies on the evolution of wing dimorphism in water striders that have assumed wing dimorphism has the potential to evolve. Our study not only demonstrates the evolvability of wing dimorphism itself, but also the evolvability of its reaction norm in response to temperature. This result will be critically important as we move forward with studies of the evolution of phenotypic plasticity in wing dimorphism in water striders and other wing dimorphic species.

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Chapter 2

Modeling the evolution of phenotypic plasticity in resource allocation in wing dimorphic insects

2.1 Introduction

How optimal allocation strategies change in response to resource availability in any given ecological situation is a neglected, yet potentially very important area of biology. Organisms acquire a fixed amount of energy from their environment that they then allocate to various structures and functions. Competing demands among traits for this resource will cause selection to favor the evolution of allocation patterns. Because traits are assumed to be subject to trade-offs that constrain their evolution, these allocation trade-offs are central to our understanding of trait evolution. However, despite the theoretical expectation of ubiquitous trade-offs, many studies have failed to detect predicted trade-offs in natural populations (for reviews see Bell and Koufopanou 1986; Reznick 1985; Stearns 1989; Reznick et al. 2000; Roff 2000, 2002).

In a landmark paper, van Noordwijk and de Jong (1986) developed a theory to explain this perplexing discrepancy between evolutionary theory and experimental data. Their theory, now generally referred to as the Y model, contends that the relative amount of variation in acquisition and allocation can explain the failure to detect a trade-off when one actually exists. When the variation among individuals in a given population is higher for allocation than for acquisition, the traits are negatively correlated, and a trade-off is detected. However, if acquisition varies more than allocation, some individuals will be better overall than others and a positive relationship will exist between the traits. Therefore, the relative amount of variation in acquisition versus allocation of resources is critical in determining whether a trade-off will be detected. It follows that any environmental variation in the amount of resources individuals are able to acquire will influence observed trade-offs in natural populations. Given that environmental variation in the availability of resources is often assumed to be great (Bashey 2006), this source of variability has the potential to substantially shift the trade-off relationship.

Several studies have shown that trade-offs can shift in response to resource availability and can even change from a negative to a positive relationship (Bell and Koufopanou 1986; Gebhardt and Stearns 1988; Kaitala 1991; Stearns et al. 1991; Leroi et al. 1994; Czesak and Fox 2003; Messina and Fry 2003; Blanckenhorn and Heyland 2004; Ernande et al. 2004; Sgrò and Hoffmann 2004). Further development of the Y model by Roff and Fairbairn (2007) has shown that the predicted relationship depends on both the mean and the variance of acquisition and allocation. Therefore, any shifts in the optimal allocation strategy in response to environmental variability in resource availability also have the potential to change the observed trade-off relationship. For these reasons, studies of the evolution of phenotypic plasticity in allocation in response to variability in resource availability have major implications for our understanding of the evolution of trade-offs in general.

Commonly, resource levels vary spatially and temporally both within and across generations, leading to variation in the amount of energy available to individuals. When faced with reduced resources, individuals could maintain the same relative proportion allocated to each trait and simply allocate fewer resources to all traits. Alternatively, individuals could exhibit phenotypic plasticity in resource allocation and alter the relative amount of resources allocated to one function versus others. For example, many species show a relative increase in resources allocated to maintenance and survival when faced with food restriction (e.g., Kaitala 1991; Chippendale et al. 1993; Ellers and van Alphen 1997; Ruf et al. 2006). If the optimal allocation strategy varies depending upon the resource environment, organisms should evolve the ability to alter their allocation strategies in response to variation in environmental resource levels, subject to the degree of predictability of this variation. In other words, they should evolve a phenotypically plastic allocation strategy. While there are many examples of phenotypically plastic allocation strategies in a wide variety of organisms (Table 2.1), demonstrating definitively that these plastic strategies are adaptive is difficult and is frequently presumed rather than proven (exceptions include Calow and Woollhead 1977; Blanckenhorn 1998; Bashey 2006).

One reason for this difficulty is a lack of specific theoretical predictions. Many general models examine both the evolution of phenotypic plasticity (Via and Lande 1985; Kawecki and Stearns 1993; DeWitt et al. 1998; Scheiner 1998; de Jong 1999; Sasaki and de Jong 1999; de Jong and Behera 2002; Ernande and Dieckmann 2004) and optimal energy allocation (Parker and Begon 1986; Perrin and Sibly 1993; McNamara and Houston 1996; Shertzer and Ellner 2002; Roff 2002), but few provide specific predictions for how plasticity in resource allocation will evolve in response to variation in resource level (exceptions include Stearns and Koella 1986; Houston and McNamara 1992; Fischer et al. 2009). Models focused on the evolution of phenotypic plasticity usually examine the general conditions favoring the evolution of a plastic phenotype over a fixed phenotype. However, the evolution of phenotypic plasticity in resource allocation in response to environmental variation in resources depends on the details of the

Table 2.1: Empirical examples of phenotypic plasticity in allocation in response to resource availability.

Organism	**Species**	**Traits**	**Reference**
Insects	*Drosophila mercatorum*	Developmental Time and Weight at Eclosion	Gebhardt and Stearns 1988
	Gerris spp.	Reproduction and Longevity	Kaitala 1991
	Drosophila melanogaster	Reproduction and Longevity	Chippindale et al. 1993
	Asobara tabida	Reproduction and Longevity	Ellers and van Alphen 1997
	Scathophaga stercoraria	Growth, Development, and Body Size	Blackenhorn 1998
	Stator limbatus	Egg Size and Egg Number	Czesak and Fox 2003
	Callosobruchus maculatus	Reproduction and Longevity	Messina and Fry 2003
Reptiles	*Microlophus delanonis*	Egg Size and Egg Number	Jordan and Snell 2002
Fish	*Poecilia reticulata*	Offspring Size and Offspring Number	Bashey 2006
Mammals	*Glis glis*	Reproduction and Longevity	Ruf et al. 2006
Cladocerans	*Daphnia magna*	Growth and Reproduction	Yampolsky and Ebert 1994
	Daphnia spp.	Growth and Reproduction	Tessier et al. 2000
Bivalves	*Anodonta piscinalis*	Maintenance, Growth, and Reproduction	Jokela and Mutikainen 1995
	Crassostrea gigas	Survival, Growth and Reproduction	Ernande et al. 2004
Plants	*Lychnis flos-cuculi*	Vegetative and Generative Reproduction	Biere 1995
Flatworms	*Dendrocoelum lacteum*	Reproduction and Longevity	Calow and Woollhead 1977
	Polycelis tenuis		
	Dugesia lugubris		

model. For example, some models of the evolution of phenotypic plasticity include variation in habitat quality and find that selection has a greater effect in “good” habitats, because these habitats produce more individuals than “bad” habitats (Houston and McNamara 1992; Kawecki and Stearns 1993). However, other models have shown that, if the plastic response to habitat quality influences competitive ability, selection will be strongest in “bad” habitats and will, therefore, have the greatest effect on the resulting reaction norm (Sasaki and de Jong 1999; Ernande and Dieckmann 2004).

These disparate results clearly indicate that the nature of habitat variability itself can have an impact on the evolved strategy. Therefore, while there are also many models of optimal allocation in various constant conditions (e.g., low and high resource availability), it is not necessarily appropriate to assume a simple reaction norm resulting from separate optima in the two environments. In fact, the resulting reaction norm has been found to depend upon both the quality and the frequency of habitats experienced (Houston and McNamara 1992; Kawecki and Stearns 1993). Consequently, despite the observed widespread occurrence of phenotypic plasticity in response to resource levels (Table 2.1), theoretical models predicting how the optimal allocation reaction norm should evolve are lacking. Thus, the assumption that this plasticity has evolved lacks theoretical justification.

The primary goal of this paper is to fill this gap by predicting the evolved allocation reaction norm in response to varying acquisition regimes in a model system for studying trade-offs, namely the evolution of wing dimorphism in insects, which represents a specific case of the evolution of migration. In this case, the optimal allocation between migratory ability and other fitness-related traits such as fecundity will vary because these insects migrate among habitats of varying quality. An obvious interaction exists between the nature of environmental variation and the evolution of plasticity in allocation. By focusing on the fundamental trade-off between migration and reproduction in these insects, we can better understand how phenotypic plasticity in allocation patterns evolves in general in migrating species.

Wing dimorphic insects exhibit a migration dimorphism with a volant (macropterous) morph and a flightless (sedentary) morph. The volant morph enjoys the obvious benefits of long-range migration, while the sedentary morph has a higher reproductive output (for reviews see Harrison 1980; Roff 1986; Dingle 1996; Zera and Denno 1997). Therefore, in general terms, these models explore how environmental variation in resource levels influences the evolution of allocation to migration versus reproduction. Migration is particularly important in heterogeneous environments but is often energetically expensive, making high demands on an individual's energy budget (Harrison 1980; Roff 1984; Roff 1986; Dingle 1996; Zera and Denno 1997; Zera et al. 1997; Crnokrak and Roff 2002). When spatiotemporal variation in resource levels is present, selection is likely to favor allocation patterns to migratory ability that differ between favorable versus unfavorable resource environments.

Roff (1994a) hypothesized that the predictability of the environment will determine the pattern of plasticity in allocation. If current resource levels are a good predictor of future resource levels, individuals in good habitats should maximize their fecundity by becoming flightless and allocating all resources to reproduction. Given that the environment is predictable, conditions will likely remain good, and their offspring will also experience a good habitat. If individuals are in a poor habitat, it is advantageous to become a volant morph and allocate resources to flight capability to escape to a better habitat given that conditions are likely to remain poor in the next generation. If resource levels are unpredictable, the opposite pattern is expected. Given that current resource levels do not predict future resource levels, individuals in poor habitats should sacrifice migratory ability and become flightless in order to allocate all resources to reproduction and maintain a non-zero fitness. However, individuals in high resource habitats should take advantage of those good conditions and become a volant morph as they can allocate resources to flight while still maintaining a relatively high fecundity. Selection will always favor the production of some volant individuals or the lineage will die out when the habitat goes extinct. We tested this prediction using two quantitative genetic simulation models.

Focusing on allocation specifically as a genetic trait, we modeled the evolution of plasticity in this trait in response to spatiotemporal variation in resources.

2.2 Study System

Wing dimorphic insects have been studied extensively and have emerged as a model system for the study of trade-offs (Harrison 1980; Roff 1986; Dingle 1996; Zera and Denno 1997). In these insects, two discrete morphs exist, a macropterous, flight-capable morph with a fully developed and functional flight apparatus and a flightless morph that is either micropterous, with greatly reduced wings, or apterous, completely lacking wings. The ability to fly confers the fitness benefit of long-range migration, which may be critical for persistence in a heterogeneous environment. Both comparative and theoretical studies have found that in habitats that are ephemeral, macropters will be favored due to their ability to colonize new habitats and escape deteriorating ones (Roff 1974a, b; Vespäläinen 1978; Harrison 1980; Roff 1994a; Dingle 1996). Given the advantage of flight capability, the persistence of flightless individuals in the population implies that some cost is associated with the ability to fly. Even in the absence of flight, making and maintaining the large flight muscles incurs a significant energetic cost (Harrison 1980; Roff 1984; Roff 1986; Dingle 1996; Zera and Denno 1997; Zera et al. 1997; Crnokrak and Roff 2002). Many studies on various wing dimorphic species have demonstrated a trade-off between flight capability and reproduction (for reviews see Harrison 1980; Roff 1986; Dingle 1996; Zera and Denno 1997), and these studies provide a strong argument that these two traits are in a functional trade-off and compete for resources. This conclusion has been confirmed by physiological studies demonstrating differences in the allocation of available nutrients in flight-capable compared to flightless morphs (Zera et al. 1994; Zera and Denno 1997; Zera and Brink 2000; Zera 2005; Zhao and Zera 2006).

Wing dimorphic insects have two allocation “decisions” to make with respect to the migration/fecundity trade-off. First, nymphs develop into either flight-capable or flightless morphs. Second, once they are adults and have a fixed wing morph, individuals allocate some proportion of their resources to flight capability and some proportion to reproduction. Wing morphology has been found to be phenotypically plastic in response to temperature (Roff 1986; Roff and Shannon 1993; Sasaki et al. 2002; Braendle et al. 2006; Fairbairn and King 2009), photoperiod (Vespäläinen 1978; Zera and Tiebel 1991; Roff 1994b; Sasaki et al. 2002), crowding (Denno et al. 1985; Harada et al. 1997; Sasaki et al. 2002; Braendle et al. 2006), food quality (Denno et al. 1985; Braendle et al. 2006), and stress (Zera and Tiebel 1988; Shimizu and Masaki 1993; Fairbairn and Yadlowski 1997) in various insects (for reviews see Harrison 1980; Masaki and Shimizu 1995; Dingle 1996; Zera and Denno 1997). Additionally, evidence exists for phenotypic plasticity in the proportion of resources allocated to flight capability versus reproduction in response to resource level and that these patterns vary among wing dimorphic species (Kaitala 1991). Phenotypic plasticity clearly has an important role for this fundamental trade-off. Therefore, this system is well suited for an exploration of the theoretical aspects of the evolution of phenotypic plasticity in allocation patterns in response to variation in resource levels.

2.3 Model Description

The models described here extend a previous model by Roff (1994a), which examined the evolution of wing dimorphism as a function of habitat persistence. For simplicity, Roff’s model ignored phenotypic plasticity in wing morph induction. It also did not explicitly model the proportion of resources allocated to each function. The present models include these elements, using this fundamental trade-off to study how phenotypic plasticity in allocation patterns evolves at the level of both wing morph induction and proportional allocation to flight ability versus reproduction. We modeled wing morph induction and proportional allocation explicitly as traits and followed their evolution in an environment with spatiotemporal variation in resource levels. Descriptions of all model parameters and values are given in Table 2.2. For all parameters, we chose fixed values designed to span a wide range of the parameter space. We ran all combinations of the chosen fixed parameter values for a total of 6,336 combinations, representing an extensive examination of the available parameter space. The model was programmed in R (v. 2.9.0; R Development Core Team 2009). A detailed description of the model can be found below.

2.3.1 The Environment

Patches and Persistence Time

Following Roff (1994a), the environment consisted of a set of n discrete patches with set persistence time T. Once a patch reached the set persistence time, the patch was eliminated and the population in that patch went extinct. The number of patches was the same as the persistence time such that each generation, one patch was eliminated and one patch was added. The patches therefore had a constant age structure with patches aged 1, 2, 3, $\ldots T$ in each generation. Roff (1994a) confirmed that the evolution of wing morph frequency is a function of the patch persistence time (T) and not the number of patches in the environment. He also considered the case of a variable persistence time and found only a small effect on the equilibrium proportion of macropterous individuals. For this reason, and because a constant persistence time and a constant age structure greatly simplify the model, we consider only these conditions. We chose persistence time values of 3, 5, 10 and 20 generations as these persistence times have been shown to produce a range of the proportion of macropterous individuals from 5% to 100% (Roff 1994a).

Table 2.2: List of parameters and their chosen values in the simulation model.

Parameters	**Description**	**Values**
Environment		
n	Number of Patches	3, 5, 10, 20
T	Persistence Time	3, 5, 10, 20
μ_r	Mean Resource Level	6, 8
σ_r	Spatial Standard Deviation of Resource Level	1, 3
r	Temporal Autocorrelation	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 0.99
Genetic Parameters		
h^2	Heritabilities of traits	0.5
r_g	Genetic correlations between traits	0
V_a	Genetic Variances	1 (a and b), 0.1 (g and z)
a_0	Initial additive genetic mean for trait a	`qnorm`(p_{mac})
b_0	Initial additive genetic mean for trait b	0
g_0	Initial additive genetic mean for trait g	0.5
z_0	Initial additive genetic mean for trait z	0
p_{mac}	Intitial proportion macropterous	0.2, 0.5, 0.8
Induction Model		
p	Proportion allocated to flight	0.25, 0.5, 0.75
s	Probablity of surviving migration	0.5, 0.9
m	Average probability of a macropter migrating	0.5, 0.9
Allocation Model		
c	Inherent cost to being macropterous	0, 0.15, 0.30
s	Probablity of surviving migration	0.5, 0.9
p_{max}	The proportion a macropter would have to allocate to flight to ensure migration	0.2, 0.5, 0.8

Spatio-temporal Variation in Resource Levels

To create spatial variation in resource level available to each individual, each patch was assigned a resource level, $R_{k,t}$ where k refers to patch and t to generation. $R_{k,t}$ was drawn from a normal distribution with mean μ_r and standard deviation σ_r. The parameter $R_{k,t}$ defines the total resource pool available to each individual in its native patch independent of population density. We manipulated the degree of spatial variation in resource levels by varying the mean (μ_r) and standard deviation (σ_r) of the distribution. We chose values for μ_r (6 and 8) that lead to widespread persistence of the population even when composed mostly of macropters. The evolution of phenotypic plasticity depends on the presence of variability in the environment. To examine the effect of the degree of spatial variability, we set σ_r to the standard value of 1 or a high value of 3. If the value pulled from the distribution was less than zero (less than 2% of trials), it was set to zero. To ensure a symmetric distribution, an upper limit was also placed at the value above the mean that was the same distance above the mean as from the mean to zero.

Temporal variation in resource levels within patches was created with a temporal autocorrelation parameter, r. A high autocorrelation causes current resource levels to be a good predictor of future resource levels. A low autocorrelation causes a low predictability of future resource levels within patches. We chose a range of autocorrelations from completely unpredictable (0) to nearly deterministic (0.99). Thus, for each patch in each generation, $R_{k,t}$ is drawn from a multivariate normal distribution defined by a matrix of means and a variance-covariance matrix (`mvrnorm` function in R). Each mean in the matrix of means defines the mean resource level for that generation, which was constant in this model (μ_r). The diagonal of the variance-covariance matrix defines the spatial variance among patches in each generation (σ_r^2). The covariances between sets of patches in any two generations f and g are equal to the variance (σ_r^2) multiplied by the correlation between generation f and generation g, which can be calculated in the following way:

$$r_{f,g} = r^{|g-f|}$$

where $r_{f,g}$ is the correlation between any two generations within a patch and r is the set auto-correlation between two successive generations within a patch. Thus, for example, in a three patch universe the variance-covariance matrix is defined as

$$\begin{pmatrix} \sigma_r^2 & r\sigma_r^2 & r^2\sigma_r^2 \\ r\sigma_r^2 & \sigma_r^2 & r\sigma_r^2 \\ r^2\sigma_r^2 & r\sigma_r^2 & \sigma_r^2 \end{pmatrix} = \sigma_r^2 \begin{pmatrix} 1 & r & r^2 \\ r & 1 & r \\ r^2 & 1 & 1 \end{pmatrix}$$

2.3.2 Quantitative Genetics of Allocation

All of the traits allowed to evolve in this model were modeled using the standard quantitative genetic approach. Accordingly, the phenotypic value of each trait was determined by both a genetic and an environmental component in the following way (Roff 1997; Roff and Fairbairn 2007):

$$Z_P = Z_A + Z_E$$

where Z_p is the phenotypic value, Z_A is the additive genetic component with a mean of μ_a and Z_E is the environmental component with a mean of zero. We modeled multiple traits and therefore, values for Z_A and Z_E were generated independently using multivariate normal distributions. The component means and the associated variance-covariance matrix define these distributions. The variance-covariance matrix is calculated from the parameter values for heritability (h^2) and the genetic and phenotypic correlations between the traits (r_A and r_P, respectively; Table 2.2).

Wing Morphology

In this model, wing morphology was assumed to be under polygenic control and was modeled as a threshold trait. When wing morphology is determined by many loci, the threshold model assumes that the discrete trait is the result of an underlying, continuously distributed trait termed the liability (Roff 1996). Individuals with liabilities above a certain threshold level develop into the macropterous wing morph (the potential migratory morph) and those with liabilities below that level develop into the flightless wing morph. The influence of environmental variation in resource level on induction of wing morphology was modeled by incorporating an environmental effect as well as a genotype by environment interaction such that genotypes vary in their response to resource level. Liability was a function of two polygenic traits, a_i, which is independent of the environment, and b_i, which determines the response to the resource level in the patch, where i refers to individual.

$$\text{Liability} = a_i + b_i \left(\frac{R_{k,t}}{\mu_r} \right)$$

where a_i and b_i are normally distributed with a mean of μ_a and 0, respectively, and variances of one. The initial value for μ_a was set to produce an initial proportion of macropterous individuals in the population (p_{mac}). If the threshold value is assumed to be zero, then μ_a corresponds to the ordinate on a standardized normal distribution at which the proportion of values above this ordinate equals p_{mac} (this value can be found using the `qnorm` function in R). The wing morphology for each individual was then determined by assigning all individuals with a liability above the threshold level (0) as macropterous and those with liabilities below the threshold level as flightless morphs. Thus, changes in the mean liability over time will

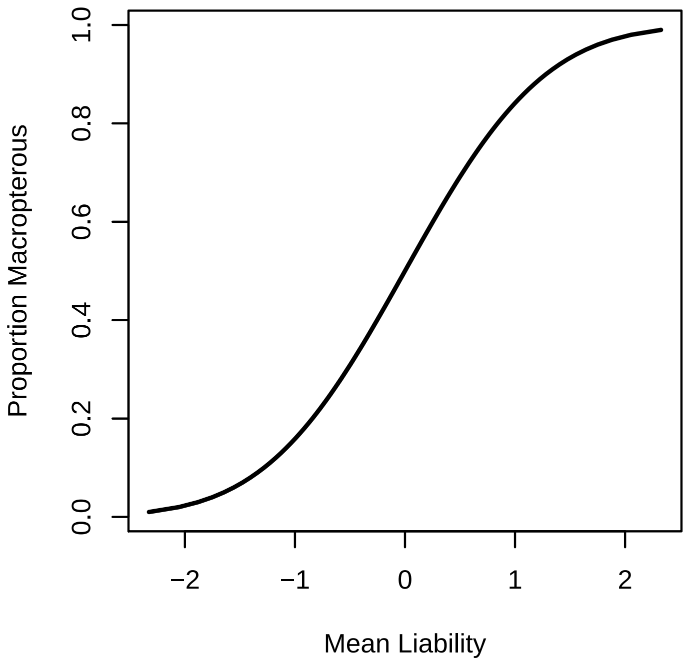

Figure 2.1: The relationship between the mean liability and the resulting proportion macropterous.

change the proportion of macropterous individuals in the population (Figure 2.1). This can occur through changes in a_i, which will change the overall liability independent of the environment or changes in b_i, which determines how liability responds to changes in resource level. An increase in a_i, b_i, or $R_{k,t}$ increases liability and hence increases the proportion of macropterous individuals, while an increase in μ_r decreases liability and hence decreases the proportion of macropterous individuals.

Proportional Allocation

As previously defined, an individual's available resource pool was equal to the resource level in that patch, $R_{k,t}$. Flightless morphs allocate all resources to fecundity whereas macropterous individuals allocate resources to both flight capability and fecundity. Resource allocation to flight capability versus fecundity in macropters was modeled as a basic Y model, where a proportion, p_i ($0 \leq p_i \leq 1$) was allocated to flight capability and (1-p_i) was allocated to fecundity. Resource allocation was modeled in two ways to create two separate models, one focused only on plasticity in wing morph induction (hereafter referred to as the induction model) and a second model focused on plasticity in both wing morph induction and resource allocation (hereafter referred to as the allocation model). These models are described separately below.

The Induction Model: Constant p In the model examining the evolution of plasticity in wing morph induction only, all macropterous individuals allocated a set proportion of their resources to flight. The parameter p determined the proportion of resources each macropter allocated to flight and remained constant within individual runs of the model; i.e., it was not allowed to evolve. We chose a range of values (0.25, 0.5, and 0.75) for this parameter.

The Allocation Model: Evolving p_i Alternatively, p_i can be modeled as a composite polygenic trait. In this case, the phenotypic value of an individual was determined by a trait independent of the environment, g_i, and a trait determining the response to the resource level in the patch, z_i in the following way:

$$p_i = g_i + z_i \left(\frac{R_{k,t}}{\mu_r} \right)$$

where g_i and z_i are normally distributed with means of 0.5 and 0, respectively, and a variance of 0.1. The initial mean was set at 0.5 to correspond to 50% allocation to flight and 50% allocation to reproduction. Given that p_i is constrained to vary between 0 and 1, the threshold model can be used with threshold values set at 0 and 1 to constrain allocation to lie within these bounds (Roff and Fairbairn 2007). The variance of g_i and z_i was set at 0.1 instead of the standard 1 to accommodate the narrow phenotypic range of 0-1: a variance of 1 produces many values outside of this range at any mean value of p_i. Evolution of p_i is a consequence of the evolution of g_i and z_i, specifically the changes in the mean values of these two traits.

If p_i is allowed to evolve to 0 (no allocation to flight in macropters), macropterous individuals are, in the above formulation, identical to flightless individuals. However, it seems reasonable to suppose that, in general, there is an inherent cost to being a macropter. We incorporated this cost by changing the threshold level from 0 to c. Here, there was a minimum amount ($0 \leq c \leq 1$) that macropterous individuals must allocate to flight capability. In the allocation model, this parameter was set to 0, 0.15, or 0.30.

2.3.3 Migration

Only macropterous individuals were able to migrate to other patches, but not all macropterous individuals migrated. The probability that an individual migrated depended on the total amount of resources devoted to flight capability (Figure 2.2). The total resources allocated to flight (f_i) depended upon the size of an individual's resource pool and the proportion (p_i) they allocated to flight.

$$f_i = p_i \cdot R_{k,t}$$

The probability of an individual migrating (m_i) increases linearly with an increasing amount of resources devoted to flight until a set maximum value (f_{max}) is reached, above which the probability of migrating remains constant at 0.99.

$$m_i = \frac{f_i}{f_{max}}$$

The parameter f_{max} was determined differently in the two models (Figure 2.2).

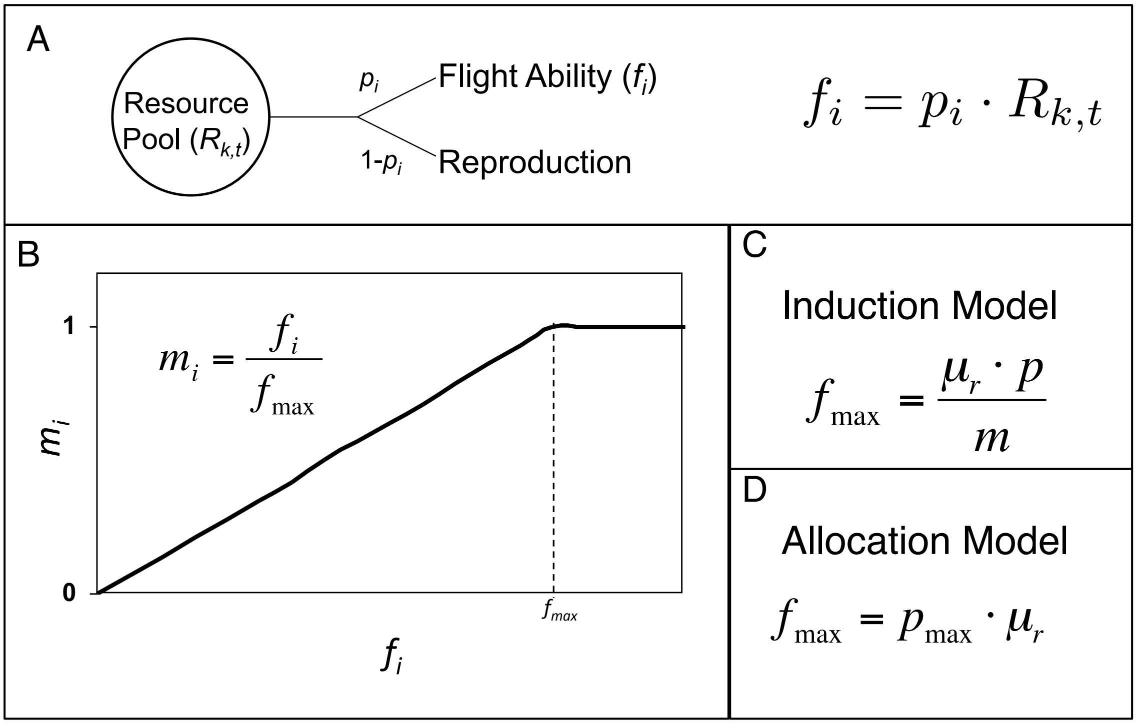

Figure 2.2: A) Schematic of the amount of resources allocated to flight ability and reproduction. The amount allocated to flight ability (f_i) is determined by the resource level in the patch ($R_{k,t}$) multiplied by the proportion that an individual allocates to flight ability (p_i). B) The relationship between the amount an individual allocates to flight (f_i) and the probability an individual will migrate. The probability of migrating increases linearly with an increasing amount allocated to flight up to a maximum value (f_{max}), above which the probability of migrating is constant at 0.99. C) The equation that determines f_{max} for the induction model. μ_r is the mean resource level in the environment, p is the proportion macropters allocate to flight, and m is the average probability an individual will migrate. D) The equation that determines f_{max} for the allocation model. μ_r is the mean resource level in the environment, and p_{max} is the amount macropters need to allocate to flight to assure migration on average.

For the induction model, the average probability that a macropter would migrate was set at a constant value (0 $\leq m \leq$ 1), which in the present analysis was either 0.5 or 0.9. The value for f_{max} was therefore set such that the average probability an individual would migrate was equal to m. This value depended on the average probability of migrating (m), the average resource level (μ_r) and the proportion macropters allocate to flight (p) in the following way (Figure 2.2):

$$f_{max} = \frac{(p \cdot \mu_r)}{m}$$

In the allocation model, we could not set the average probability of migrating because this depended upon the proportion of resources that macropters allocated to flight, which was allowed to evolve in this model. Therefore, we set the proportion a macropter would have to allocate to flight to ensure migration (m = 0.99) on average. In the allocation model, this parameter was set as 0.8, 0.5 or 0.2. This parameter (0 $\leq p_{max} \leq$ 1) determined fmax in the following way (Figure 2.2):

$$f_{max} = p_{max} \cdot \mu_r$$

A proportion s (0 $\leq s \leq$ 1) of migrating insects was not successful in surviving and finding a new patch. The energetic cost of actual flight was absorbed into this survival cost as in Roff (1994a). In this model, s was set to 0.5 or 0.9. Migrants entered a common migrant pool, and surviving migrants were distributed randomly among patches.

2.3.4 Reproduction

Mating

Individuals mated randomly within patches preceding migration. The mean additive genetic value of offspring for each of the traits a_i, b_i, g_i, and z_i is given by:

$$\frac{\text{female breeding value} + \text{male breeding value}}{2}$$

Relative fitness

Flightless morphs devoted all their resources to reproduction and therefore, their fecundity was a function of the resource level in the patch:

$$\text{Fecundity} = R_{k,t}$$

For macropters, fecundity was a function of both resource level and the proportion of resources devoted to fecundity versus flight capability:

$$\text{Fecundity} = F_{i,k,t} = R_{k,t}\left(1 - p_i\right)$$

The relative fitness of an individual female is given by:

$$W_i = \frac{\text{Individual Fecundity}}{\text{Total Patch Fecundity}}$$

Non-migrants reproduce in their natal patch. Successful migrants reproduce in a single new patch. In both models, the carrying capacity, K was set at 300 individuals for each patch.

While there is evidence for a cost associated with macroptery in males in some systems (Tanaka 1999; Crnokrak and Roff 2000; Langellotto et al. 2000), the majority of research has focused on the trade-off in females (see section 2.2 Study System and references therein; page 46), and thus, we have much more information about this trade-off in females. For this reason, we focused here only on the evolution of allocation in females in this model. The mean values for wing morphology and allocation in males simply tracked the population mean and all males had equal fitness.

2.3.5 Time Step

The basic time step was a generation. After each generation, the resource level in the patch changed according to the temporal autocorrelation. The patches increased in age and those over the set persistence time T, were eliminated. We followed population means across the generations. Each simulation continued for 1,000 generations to ensure the equilibrium additive genetic values for a_i, b_i, g_i, and z_i were reached.

2.3.6 Evolved Reaction Norm

For the reaction norm of the proportion of individuals that are macropterous (hereafter proportion macropterous) in response to resource level, we determined the evolved reaction norm slope by regressing the proportion macropterous produced within the patches against the resource levels in those patches for the last 100 generations. The evolved reaction norm slope was estimated as the slope of the resulting best fit line. The same procedure was used to determine the evolved reaction norm slope for the proportion allocated to flight ability in response to resource level. The mean proportion allocated to flight ability by macropters was regressed against the resource level in the patches for the last 100 generations of the simulation and the slope of the best fit line was taken as the evolved reaction norm slope (Figure 2.3).

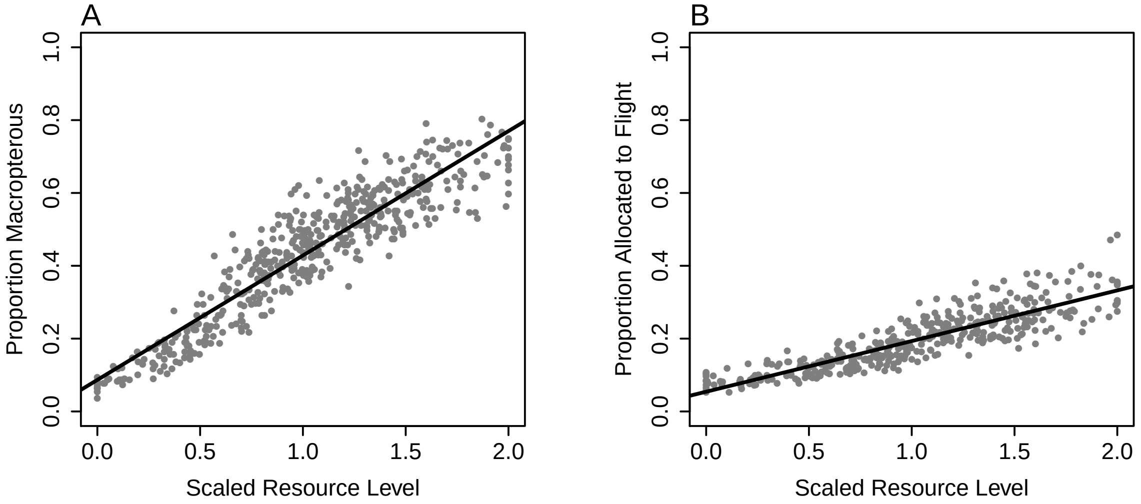

Figure 2.3: Examples of the A) proportion macropterous (T = 10, μ_r = 6, σ_r = 3, r = 0, p_{mac} = 0.2, p = 0.25, s = 0.9, m = 0.9) , and B) proportion allocated to flight ability (T = 5, μ_r = 6, σ_r = 3, r = 0, p_{max} = 0.4, c = 0, s = 0.9) in response to the resource level scaled to the mean resource level (μ_r) for the last 100 generations of the simulation run. Solid lines indicate the line of best fit. The slope of the best fit line is equal to the evolved reaction norm slope.

2.4 Results

2.4.1 Proportion Macropterous

Induction Model

We could successfully predict the equilibrium proportion of macropterous individuals independently of the evolved pattern of phenotypic plasticity. An average proportion of macropterous individuals was found across all the resource levels that evolved for the parameters above, independent of wing morphology changes associated with resource level. Roff (1994a) found that the equilibrium proportion of macropterous individuals in the population could be predicted by four parameters: the persistence time of patches (T), the cost of being winged (the proportion of resources devoted to flight, p), the proportion of the population that migrates

(m), and the proportion of migrants that survive migration (s). These four parameters predicted the proportion macropterous using the following equation (Roff 1994a):

$$\text{Proportion Macropterous} = \ln\left(T\left((1-p)\cdot(1-m+ms)\right)\right)$$

We expected these same parameters to determine the overall equilibrium proportion of macropterous individuals in our model independent of the evolved pattern of plasticity in wing morphology. This prediction was confirmed using a linear regression of the natural log of the evolved proportion macropterous and the calculated value from the above equation from Roff (1994a). The linear regression accounted for 97% of the variance in the overall proportion macropterous in the population ($R^2 = 0.97$, $P < 0.001$, Figure 2.4) demonstrating a very high degree of predictability. The equilibrium proportion macropterous was independent of the temporal autocorrelation and of the resulting pattern of plasticity in wing morphology.

Allocation Model

In the allocation model, the equilibrium proportion macropterous was strongly influenced by the cost of macroptery (c). When there was no cost of macroptery and p_i was allowed to evolve to zero, the resulting proportion macropterous was always very high and often equal to one (all macropterous individuals). As the cost of macroptery increased, there was a wider distribution of equilibrium proportion macropterous individuals (Figure 2.5).

2.4.2 Phenotypic Plasticity

To determine which parameters in the models were most important in predicting the evolved reaction norm slope for both the induction of wing morphology and the proportional allocation of resources, we performed a multiple regression analysis. We focused only on the independent additive contribution of each variable in this first analysis and therefore did not

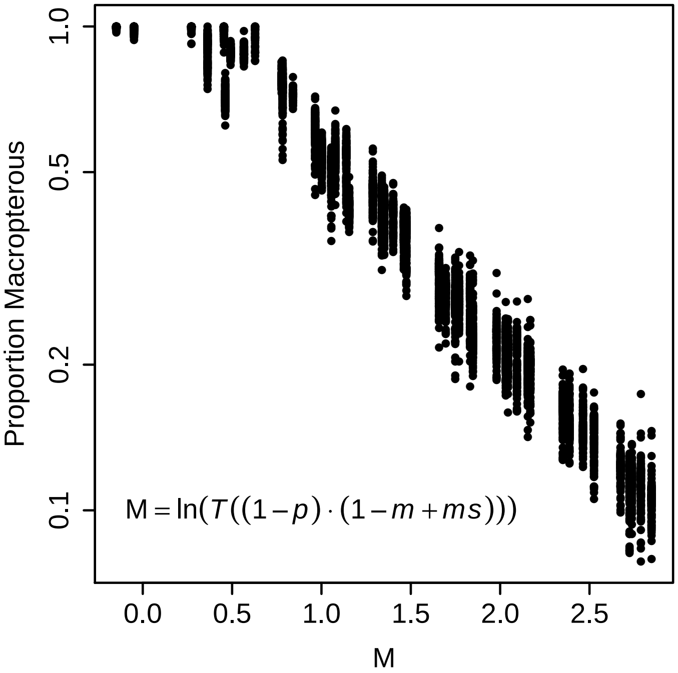

Figure 2.4: The proportion macropterous versus the equation predicting the optimal proportion macropterous. M = the predicted proportion macropterous, T = persistence time, p = the cost of flight ability, m = the proportion migrating, and s = the proportion surviving migration. The y axis is a ln scale.

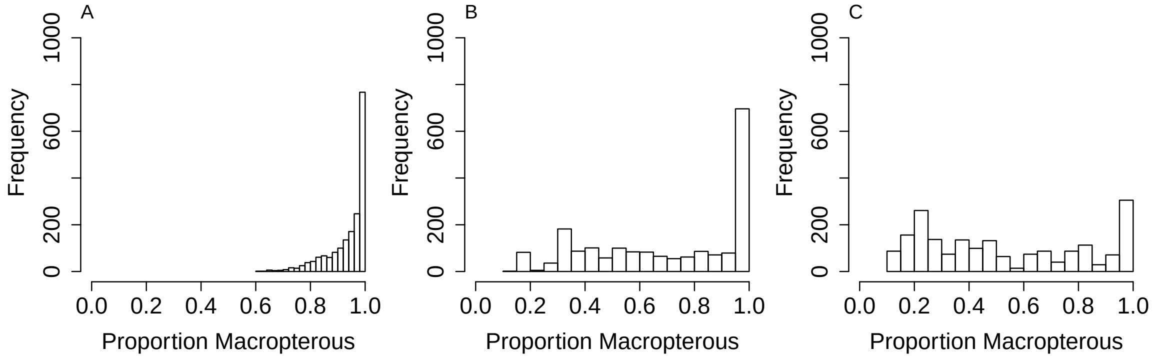

Figure 2.5: Histograms showing the frequency of the equilibrium proportion macropterous for different costs of macroptery (c). A: $c = 0$, B: $c = 0.15$, C: $c = 0.3$.

include interaction terms. Semi-partial R^2 values were calculated from standardized correlation coefficients and are reported for each of the parameters for both models in Table 2.3. These values indicate the proportion of the variance in the evolved reaction norm slope can be explained by the focal parameter once the additive effects of the other parameters are removed. As can be seen, all the parameters were highly significant: however, only the autocorrelation, r, and the standard deviation in resource level, σ_r, explained a substantial amount of the variance in the evolved reaction norm slope. This result was consistent between the models and for both wing morph induction and proportional allocation within the allocation model.

Temporal Autocorrelation

As shown above, the temporal autocorrelation, r, was consistently by far the strongest predictor of the evolved reaction norm slope. The temporal autocorrelation had a strong effect on the sign of the evolved reaction norm; as the autocorrelation increased, the slope of the reaction norm decreased. As we predicted, a low autocorrelation resulted in more positive reaction norms (increased allocation to flight in high resource environments), and a high autocorrelation resulted in more negative reaction norms (increased allocation to flight in low resource environments; Figure 2.6). This result was true in both the induction model and the

Table 2.3: Semi-partial R^2 values and *P*-values for the parameters in the induction model and the allocation model.

Parameter	**R^2**	***P*-value**
Induction Model		
T	0.52%	<0.001
μ_r	0.33%	<0.001
σ_r	9.33%	<0.001
r	22.8%	<0.001
p_{mac}	5.02%	<0.001
p	4.70%	<0.001
s	0.05%	0.03
m	0.53%	<0.001
Allocation Model		
Proportion Macropterous		
T	0.67%	<0.001
μ_r	0.68%	<0.001
σ_r	10.3%	<0.001
r	14.70%	<0.001
p_{max}	0.78%	<0.001
c	0.25%	<0.001
s	0.22%	<0.001
Proportion Allocated		
T	1.04%	<0.001
μ_r	0.58%	<0.001
σ_r	8.30%	<0.001
r	10.90%	<0.001
p_{max}	0.40%	<0.001
c	0.21%	<0.001
s	0.21%	<0.001

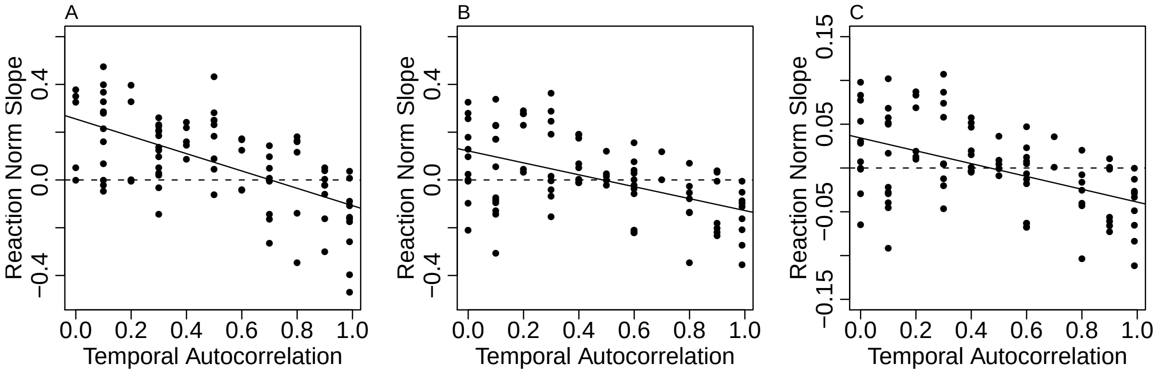

Figure 2.6: A) The evolved reaction norm slope for the proportion macropterous versus the temporal autocorrelation for 100 random parameter combinations from the induction model. B) The evolved reaction norm slope for the proportion macropterous versus the temporal autocorrelation for 100 random parameter combinations from the allocation model. C) The evolved reaction norm slope for the proportion allocated to flight ability versus the temporal autocorrelation for 100 random parameter combinations from the allocation model. Solid line: line of best fit, Dashed line: reaction norm slope of zero (no plasticity).

allocation model for both wing morph induction and proportional allocation of resources. Thus, in general, a negative correlation exists between the slope of the reaction norm and the autocorrelation.

A sensitivity analysis revealed the above pattern to be consistent across a wide range of the parameter space. For this analysis, we varied the temporal autocorrelation from 0 to 0.99 and held all other parameter values constant. We then calculated the correlation between the temporal autocorrelation and the evolved reaction norm slope. We calculated this correlation for twenty random combinations of parameter values. In the induction model, 89% were negative correlations. A χ^2 goodness of fit test confirmed that this pattern is significantly different from the null expectation of 50% negative correlations (χ^2 = 10.9, P = 0.0009). In the allocation model, the correlation between the temporal autocorrelation and the slope of the reaction norm for the proportion macropterous was negative 88% of the time and was significantly different from the null expectation of 50% (χ^2 = 9, P = 0.01). The correlation between the temporal autocorrelation and the slope of the reaction norm for the proportion allocated to flight was negative 81% of the time and was also significantly different than 50% (χ^2 = 6.5, *P* = 0.003).

Interaction between the Temporal Autocorrelation and Other Parameters

The autocorrelation clearly did not result in a negative correlation 100% of the time and, therefore, was not the only parameter that influenced the evolution of plasticity. While none of the other parameters had a large effect size in either of the models, the parameters interact with the autocorrelation to alter the evolved reaction norm. We examined this potential interaction by performing a second sensitivity analysis.

Once again we examined the correlation between the temporal autocorrelation and the evolved reaction norm slope: however in this analysis, we compared this correlation for the chosen values for each parameter. All other parameter values were held constant. We compared these correlations for 20 random combinations of parameter values for each of the parameters we examined. We then tested to see if the correlations differed using a paired t-test when the chosen parameter consisted of only two values (e.g., s) or an ANOVA followed by Tukey's pairwise comparisons when testing more than two values (e.g., three values of c). Both the spatial standard deviation of resource level (σ_r) and the persistence time (T) significantly altered the relationship between the temporal autocorrelation and the resulting reaction norm slope in both models. The other parameters did not significantly influence the relationship between the autocorrelation and the slope of the reaction norm as indicated by a lack of significant differences in the effect of varying the parameter value (Table 2.4).

We found that higher spatial standard deviation (σ_r) was associated with stronger evolved plastic responses. Environmental heterogeneity has long been known to be required for the evolution of phenotypic plasticity (Via and Lande 1985). If the environment does not vary spatially in resource level, no selection for plasticity will occur. Our results are consistent with this idea. Higher levels of spatial variation (higher σ_r) resulted in a significantly stronger

Table 2.4: Results of a sensitivity analysis examining the effects of the model parameters on the relationship between the temporal autocorrelation and the reaction norm slope.

Induction Model			Allocation Model				
				Proportion Macropterous		Proportion Allocated	
Parameter Value	y	Significance	Parameter Value	y	Significance	y	Significance
T			T				
3	-0.17	a	3	-0.16	a	-0.13	a
5	-0.62	b	5	-0.43	ab	-0.48	b
10	-0.71	b	10	-0.6	b	-0.53	b
20	-0.58	b	20	-0.4	ab	-0.37	ab
μ_r			μ_r				
6	-0.48	a	6	-0.34	a	-0.43	a
8	-0.48	a	8	-0.34	a	-0.43	a
σ_r			σ_r				
1	-0.42	a	1	-0.39	a	-0.26	a
3	-0.66	b	3	-0.56	b	-0.59	b
p_{mac}			p_{max}				
0.2	-0.54	a	0.2	-0.45	a	-0.41	a
0.5	-0.47	a	0.5	-0.32	a	-0.33	a
0.8	-0.43	a	0.8	-0.28	a	-0.37	a
p_i			c				
0.25	-0.60	a	0	-0.12	a	-0.42	a
0.50	-0.66	a	0.15	-0.44	b	-0.40	a
0.75	-0.48	a	0.3	-0.52	b	-0.54	a
s			s				
0.5	-0.51	a	0.5	-0.33	a	-0.37	a
0.9	-0.50	a	0.9	-0.32	a	-0.35	a
m							
0.5	-0.49	a					
0.9	-0.48	a					

Note: y is the correlation between the temporal autocorrelation and the reaction norm slope for the parameter values listed. Different letters in the “Significance” column indicate that y differs significantly between parameter values.

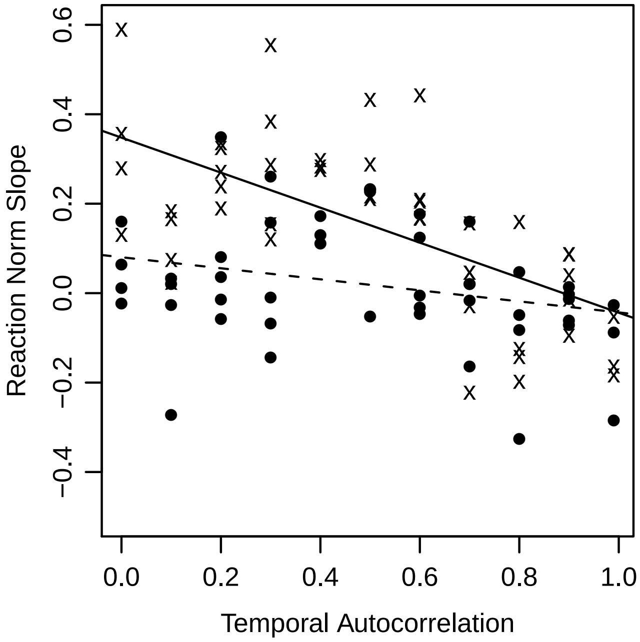

Figure 2.7: The evolved reaction norm slope for the proportion macropterous versus the temporal autocorrelation for a spatial standard deviation of 3 (x, solid line) and a spatial standard deviation of one (points, dashed line) for 100 random combinations of parameter values in the induction model.

relationship between the autocorrelation (r) and the reaction norm slope, whereas lower levels of spatial variation (lower σ_r) resulted in less plasticity overall (Table 2.4, Figure 2.7).

Persistence time (T) also significantly affected the relationship between the autocorrelation and the reaction norm slope. Persistence time was the strongest predictor of the evolved proportion macropterous (Roff, 1994a; see Proportion Macropterous on page 60). A very short persistence time will select for a very high proportion macropterous being produced in all resource environments and, therefore, very little plasticity in wing morph induction. A similar effect on plasticity exists in the proportional allocation of resources as very high or very low persistence times select for a fixed strategy. This pattern is apparent in Table 2.4 and Figure 2.8 where the weakest relationships between the autocorrelation, and the evolved reaction norm slopes were for extreme persistence times.

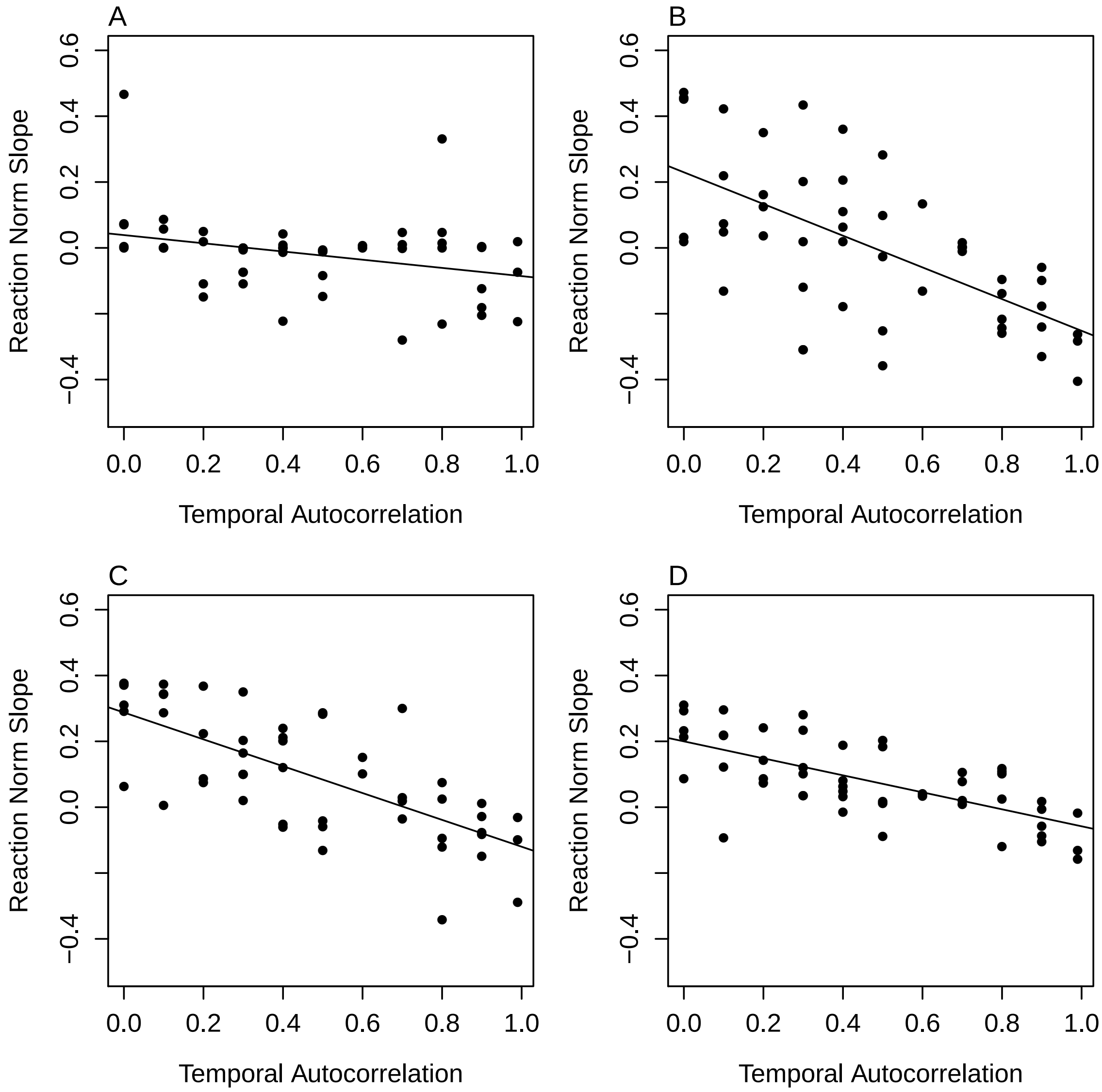

Figure 2.8: The evolved reaction norm slope for the proportion macropterous versus the temporal autocorrelation for a persistence time of A) 3, B) 5, C) 10, D) 20. Each graph contains points corresponding to 50 random parameter combinations in the induction model.

A parameter unique to the allocation model, the cost of macroptery, also significantly influenced the relationship between the temporal autocorrelation and the reaction norm slope for the proportion macropterous. Whether a cost was an inherent to being macropterous, or whether macropterous individuals were able to allocate zero to flight strongly influenced the proportion of macropterous individuals (Figure 2.5 on page 63). No cost led to fixation (or near fixation) of macroptery in the population and therefore very little plasticity in wing morph induction. Including a cost led to a wider distribution of proportion macropterous individuals and increased plasticity in the proportion of macropterous individuals. In contrast, the cost of macroptery did not significantly influence the degree of plasticity in the proportion allocated to flight (Table 2.4, Figure 2.8).

2.5 Discussion

The models described above show how and when phenotypic plasticity in resource allocation will evolve in response to variation in resource availability in wing dimorphic insects, a model system for studying trade-offs. In our models individuals were distributed in discrete patches that varied in their resource level. The essential assumptions of the models were: a) migration between patches takes place only by flight and therefore only by macropterous individuals, b) the probability that a macropterous individual will migrate is a function of the amount of resources an individual allocates to flight ability, c) macropterous individuals incur a fecundity cost that is determined by a simple Y model and is therefore a function of how much an individual allocates to flight ability, and d) only a proportion of migrants survive migration. Our models predict that the temporal autocorrelation between patches (i.e., how predictable the resource level in a patch is over time) is a key factor in the pattern of plasticity in resource allocation that evolves. This relationship was hypothesized by Roff (1994a), but our models are

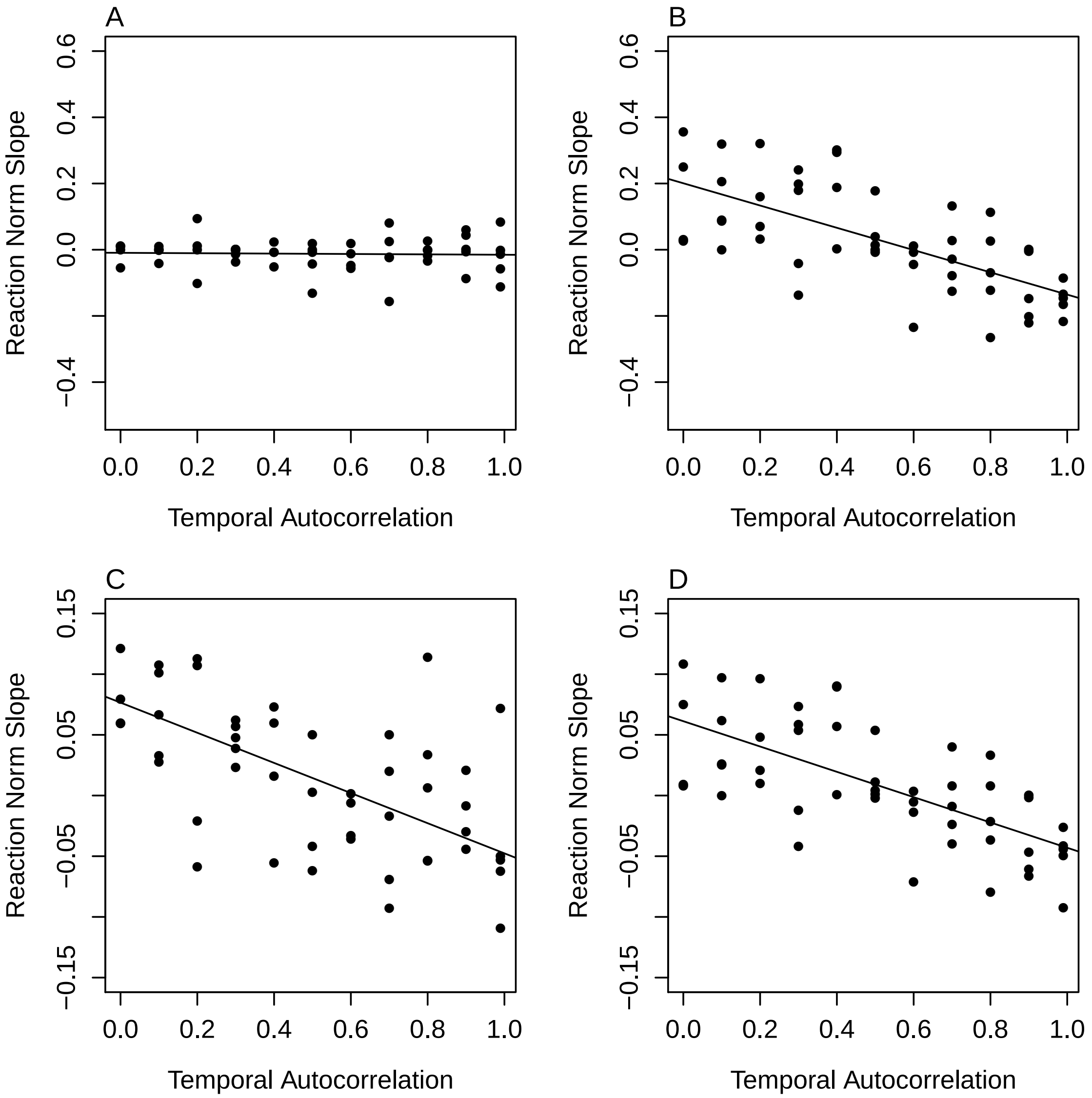

Figure 2.9: A and B) The evolved reaction norm slope for the proportion macropterous, versus the temporal autocorrelation for a cost of macroptery = 0 (A) and 0.3 (B) for 50 random parameter combinations in the allocation model. C and D) The evolved reaction norm slope for the proportion allocated to flight ability versus the temporal autocorrelation for a cost of macroptery = 0 (C) and 0.3 (D) for 50 random parameter combinations in the allocation model.

the first to show that the evolution of phenotypic plasticity in resource allocation depends upon the predictability of resource levels over time.

In environments with a high degree of predictability, the evolved reaction norm is one in which individuals allocate more to flight in low resource environments and less to flight in high resource environments. In low resource patches selection for an “escape” response is present, with individuals allocating to flight in low resource environments to escape those unfavorable conditions. The high degree of predictability indicates these habitats will likely remain unfavorable. High predictability also selects for less allocation to flight in high resource patches as these habitats are also likely to remain favorable. In contrast, in environments with low predictability across time, the models predict the opposite pattern. Here, selection favors a reaction norm where individuals in good habitats allocate more resources to flight. The model assumes these insects only move between patches by flight and hence, flight at some point is a necessary for a lineage to remain viable or it will go extinct when the patch is eliminated. Therefore, individuals in an unpredictable resource environment allocate the most to flight when resources are high. The fact that resources are unpredictable means there is no advantage to remaining in a good resource patch, as it does not indicate resources will be high in the future. In low resource environments, selection favors individuals who conserve their resources and allocate to reproduction.

We examined two types of plasticity in resource allocation: plasticity in wing morph induction (or the proportion macropterous) and plasticity in the proportion of resources allocated to flight ability. The models predict this same relationship between the temporal autocorrelation and the evolved reaction norm slope for the proportion macropterous in the induction model and for both the proportion macropterous and the proportion allocated to flight in the allocation model.

Wing dimorphic species often display plasticity in both wing morph induction and proportional allocation of resources. However, different species of insects vary in their response to resource level. For example, in aphids (Braendle et al. 2006), planthoppers (Denno et al. 1985), and lygaeid bugs (Sasaki et al. 2002), crowding induces production of the flight capable morph, a response that is often intensified by low resource levels. This response is hypothesized to be an escape response because migrants can potentially find higher quality sites for their offspring. Aphids, for example, typically have several generations on the same host plant and hence present nutritional conditions of the plant, induced by the effects of insect density, are a good indicator of conditions likely to be encountered by the next generation (Braendle et al. 2006). In contrast, in crickets high stress induces production of the flightless morph (Zera and Tiebel 1988; Roff 1990; Shimizu and Masaki 1993; Fairbairn and Yadlowski 1997). The hypothesis in this case is that insufficient resources are available to adequately support reproduction and flight capability and that present conditions are not good predictors of conditions likely to be faced by the next generation (Roff 1990; Zera and Denno 1997), which is frequently the next year (Alexander 1968).

Flight capable individuals of wing dimorphic insects also show plasticity in the proportion of resources allocated to flight capability versus reproduction in response to resource level, and these patterns vary among species. For example, waterstriders vary allocation to flight capability, reproduction and longevity in response to resource level variation at both the species and population levels (Kaitala 1991). The amount allocated to each of these functions shifts at high or low food levels, and this response differed between three *Gerris* species and between two populations of a single species, *Gerris thoracicus*. These models have the potential to explain when these contrasting patterns of plasticity might be favored for different populations. If these species/populations differ in the predictability of resource availability over time, the models indicate this will select for differing patterns of plasticity and is therefore a potential explanation for why these varying patterns exist. It is important to note, however, that our models make predictions for the evolution of plasticity in the global population. Within patches, there will be selection for increased allocation to reproduction. Therefore, when sampling within patches over time, the frequency of flightless forms is expected to increase as is allocation to reproduction.

These models also predict when a fixed strategy will be favored over a plastic strategy. Not surprisingly, we found that if there is not sufficient spatial variation in resource levels, there is not substantial evolution of phenotypic plasticity in response to resource levels. If individuals do not experience varied resource levels among patches, there won't be selection for a response to those resource levels. This result is consistent with several other models of the evolution of phenotypic plasticity (Via and Lande 1985; Scheiner 1993; Via et al. 1995). Additionally, selection for very high or low allocation to flight leads to a lower degree of plasticity. We know from Roff (1994a) that very short persistence times or very long persistence times will lead to near fixation of flight capable and flightless morphs respectively. Obviously, if selection for one morph is strong enough to drive it to fixation, there will be no plasticity. We also found this pattern when we examined proportional allocation in the allocation model. When there is selection for very high or very low allocation to flight ability because of very short or long persistence times, a lower degree of phenotypic plasticity results. Most intriguing, however, is that at intermediate levels of environment predictability, a fixed strategy was favored over a plastic strategy. This is due to the fact that opposing reaction norms are favored at the extremes of predictability and unpredictability—leading to a reaction norm with a negative slope at high degrees of predictability and a positive slope at low degrees of predictability. Therefore, at intermediate levels of predictability, we see a reaction norm with a slope near zero, or no plasticity. Field studies are needed to determine how predictable resource levels are in various populations and how often populations experience environments with high, low or intermediate levels of environmental predictability with regard to resource levels.

The allocation model differed from the induction model in that it allowed for the evolution of plasticity in the proportion of resources allocated to flight and reproduction in addition to wing morph plasticity. In this model, the proportion that individuals allocate to flight ability is a genetically determined trait that is allowed to evolve. In this case, it is possible for macropters to become indistinguishable from flightless morphs if they allocate no resources to flight ability. We wanted to also examine the case when macropters would have to allocate at least some of their resources to flight ability and, therefore, we included an inherent cost to macroptery as a parameter. This parameter strongly influenced the evolution of plasticity in wing morph induction. When there is no inherent cost to macroptery ($c = 0$), the flightless morphs lose their fecundity advantage and there is fixation (or near fixation) of macropterous individuals. This obviously leads to much less plasticity in the proportion macropterous. However, this inherent cost did not influence the degree of plasticity in proportional allocation in macropters. Some plasticity in proportional allocation for macropters is always favored, even when there is also substantial plasticity in wing morph induction as well. This fact suggests that the ability of macropters to alter their proportional allocation based on their resource environment is an important adaptation to variation in resource levels. There are relatively few studies involving wing dimorphic insects that focus specifically on plasticity in the proportion of resources allocated to flight versus reproduction by macropters (see Kaitala 1991 for an exception), which this model suggests is an important avenue for future research. Additionally, it is important to examine the case where the resource level in the patch varies between the time of wing morph induction and the time when resources are allocated between flight ability and reproduction. In this case, nymphs would induce their wing morph in a different resource environment than the environment where adults would allocate their resources between flight ability and reproduction. This situation would potentially select for greater plasticity in proportional allocation to account for changing resource levels between nymph and adult habitats.

There are several additional simplifying assumptions in these models that have implications for its results and could be the subject of future studies. In our models, we assume that wing morph induction and proportional allocation are determined by a genetic component independent of the environment and a genotype by environment interaction. Because the plastic response was only determined by a single quantitative trait, the response to the environment could only be linear. In nature, reaction norms can be complex (Via et al. 1995), and we may be missing some of this complexity by modeling the evolution of plasticity in this way. We hope to extend this model to allow for the evolution of more complex reaction norms in future work. In addition, in our models, there is no relationship between the quality of the patch and the probability the patch will go extinct. While there are many cases where patch extinction is related to catastrophic events (e.g., hurricanes, floods), there are other cases where patches deteriorate over time ultimately leading to extinction. In these cases, extinction of the patch itself becomes a predictable event and one would expect strong selection for increased allocation to flight in low resource environments. Also, in our models, the size of an individual's resource pool is determined by the resource level in its natal patch and does not change over time. In reality, resource allocation will be a dynamic process and may depend on changes in the size of one's resource pool across an individual's lifetime. We hope to incorporate this level of complexity in future work.

These models represent a case where the nature of the environmental variation—namely the degree of predictability of that variation—experienced by populations is key to the allocation strategy that evolves. The evolved allocation strategy depends on the environmental predictability so strongly in this system because individuals are able to migrate among habitats of varying quality but this migration is determined by how much an individual allocates to migratory ability versus other traits in varying resource environments. Our models are consistent with previous studies of the evolution of migration rate. The induction model showed that the proportion of macropterous individuals (which correlates with migration rate) depends on persistence time, the cost of flight ability, the migration rate and survival, consistent with Roff (1994a). While these factors determine the overall migration rate across all habitats, the model shows that the degree of environmental predictability dramatically changes the pattern of plasticity that evolves. Changes in environmental predictability change the benefits of migrating in low versus high resource environments. A recent theoretical study by Bonte and De La Peña (2009) found that increased environmental stochasticity resulted in a positive relationship between body condition and migration, consistent with our findings. In general, the evolution of phenotypic plasticity in trade-offs involving migration may depend on environmental predictability in a way not sufficiently appreciated previously.

In many systems, the population is structured as a metapopulation with individuals distributed throughout the landscape in suitable patches separated by unsuitable habitat (Hanski 1998). In these systems, movement among these patches requires individuals to allocate a portion of their resources to migratory ability. Our results indicate that, in these systems, the predictability of the habitat quality will be the major factor determining the pattern of phenotypic plasticity in resource allocation that evolves. Resources can also vary in the environment over the course of an individual's lifetime. The predictability of this kind of variation has already been shown to lead to very different reaction norms with regard to reproductive strategy (Fischer et al. 2009), and we expect environmental predictability across an individual's lifetime will consistently be important in determining the evolution of phenotypic plasticity in resource allocation.

Aside from contributing to our understanding of the evolution of phenotypic plasticity, these models contribute to our understanding of trade-offs in general. Whether a trade-off is observed at the population level is determined by the amount of variation in acquisition relative to allocation (see Introduction and references therein). Phenotypic plasticity is an important potential source of variation in these traits (Table 2.2). Our models show the evolution of environmentally induced variation in allocation in response to variation in acquisition regimes. That variation in allocation may evolve to depend upon variation in acquisition introduces a level of complexity to our understanding of the evolution of trade-offs. This complexity is an important avenue for future research. Our models have the potential to incorporate allocation of resources to acquisitive ability itself, and this would feed back to the size of an individual's resource pool. Additionally, individuals' acquisition could be influenced by the population density of the patch. In this case, allocation to acquisitive ability would correlate with competitive ability. An understanding of how the evolution of resource acquisition and subsequent resource allocation may be linked is key to understanding trade-offs in general.

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Chapter 3

Estimating energy acquisition and allocation for a life history trade-off in *Gryllus firmus*

3.1 Introduction

Resource based trade-offs entail the differential allocation of resources between competing traits and are a universal characteristic of all organisms (Roff 2002). However, when measured at the population level, the expression of trade-offs is dependent on the amount of variation in individual resource acquisition (van Noordwijk and de Jong 1986; Reznick et al. 2000). Individuals with large resource pools will have the ability to allocate a greater amount of resources to all traits when compared to individuals with small resource pools. This effect can obscure detection of a functional trade-off and was modeled by van Noordwijk and de Jong (1986). These authors showed that the relationship between two traits involved in a trade-off will be positive when the relative variation in acquisition exceeds that in allocation.

A negative relationship is observed when the relative variation in acquisition is less than the relative variation in allocation. Therefore, any study of resource based trade-offs must consider population level variation in resource acquisition in addition to differential resource allocation.

A serious challenge to any study of acquisition and allocation is reliably measuring these two variables, which requires quantifying both an individual's total resource pool and the proportion of those resources allocated to various traits. For most life history trade-offs, it is assumed that energy is the major limiting resource and in this case, total acquisition will be the total energy acquired by an organism and allocation will be the proportion of that energy allocated to various traits. Estimating energy acquisition can be challenging. Energy acquisition is a complex trait that is potentially influenced by many factors including food availability, time spent foraging, the rate of digestion and absorption of nutrients, and the efficiency of digestion and absorption of nutrients (for reviews see Ricklefs 1991; Weiner 1992; Hammond and Diamond 1997). In the past, energy acquisition has been estimated by a number of different methods including total mass (Christians 2000; Brown 2003; Uller and Olsson 2005), size at a given age (Biere 1995; Dudycha and Lynch 2005), growth rate (Tessier and Woodruff 2002), absorption efficiency of macronutrients (Zera and Brink 2000), and feeding rate (Ernande et al. 2004). Estimating allocation includes similar challenges. Studies of differential resource allocation typically do not measure allocation in units of energy. For example, a study of the trade-off between reproduction and survival might use egg number and/or egg size as an estimate for reproductive output and lifespan as a measure of survival. These types of measurements are assumed to be correlated with the amount of energy allocated to different functions. However, life history traits are complex and will often involve many factors not accounted for by these simple measurements, especially in males. A thorough study of life history trade-offs requires a more complete estimate of acquisition and allocation in units of energy.

In this study, we estimate acquisition and allocation in a model system: the trade-off between flight capability and reproduction in the sand cricket, *Gryllus firmus*. This species is one of many wing dimorphic species of insects (for reviews see Harrison 1980; Roff 1986; Dingle 1996; Zera and Denno 1997). In wing dimorphic insects, two discrete morphs exist, a macropterous, flight capable morph with fully developed functional flight apparatus (hereafter LW for "long-wing") and a flightless morph that is micropterous with greatly reduced wings (hereafter SW for "short-wing"). Numerous studies in many species have shown that LW morphs have decreased fecundity in comparison with SW morphs (Harrison 1980; Roff 1984; Roff 1986; Dingle 1996; Zera and Denno 1997) and that, even in the absence of fight, making and maintaining the large flight muscles incurs a substantial energetic cost (Harrison 1980; Roff 1984; Roff 1986; Dingle 1996; Zera and Denno 1997; Zera et al. 1997; Crnokrak and Roff 2002; Nespolo et al. 2008). Many physiological studies have demonstrated differences in the allocation of available nutrients in LW compared to SW morphs, confirming that flight capability and fecundity are in a functional trade-off and compete for resources (Zera et al. 1994; Zera and Denno 1997; Zera and Brink 2000; Zera and Larson 2001; Zera 2005; Zera and Zhao 2006).

Most studies of this trade-off in wing dimorphic insects utilize ovary mass as a proxy for allocation to reproduction and dorso-longitudinal muscle (main flight muscles, hereafter DLM) mass as a proxy for allocation to flight capability. The masses of these organs are likely correlated with allocation to reproduction and flight capability. However, because allocation is measured as a proportion, it is important that an estimate of allocation accurately reflects the relative amounts allocated to flight capability and reproduction. Ovaries are an order of magnitude larger than DLMs, but this difference does not accurately reflect the relative allocation to reproduction and flight capability.

The energy allocated to ovaries encompasses a majority of the allocation to reproduction in females of this species. *Gryllus firmus* females retain their eggs when kept as virgins, and the mass of ovaries at 7 days of age past the final ecdysis is known to correlate very highly ($r > 0.99$) to total fecundity (Roff 1994), allowing ovary mass to serve as a reliable index of fecundity. Energy devoted to producing eggs is a good estimate of allocation to reproduction in this species. *Gryllus firmus*, like most insects, do not care for their offspring and therefore there are few energy investments beyond that required to produce eggs (Gillott 1995). Therefore, if one can estimate the energy allocated to ovaries, this would likely be a good proxy for allocation to reproduction.

Allocation to flight capability is a complex trait and is not encompassed by the energy allocated to DLMs alone. Previous studies have shown that LW females have higher levels of biosynthesis of lipids and higher levels of triglycerides than SW females (Zera and Larsen 2000; Stirling et al. 2001; Zera 2005). The increased accumulation of triglycerides in the LW morph prepares it for flight, because triglycerides are the main flight fuel in Orthopterans (Gillott 1995). Macropterous (LW) females also allocate more protein and lipid to the soma, while SW females allocate more protein and lipid to ovaries (Zera and Zhao 2006). In addition to these differences in the allocation of nutrients, previous studies have suggested that large DLMs may have a high maintenance cost. Female macropters (LW) with functional DLMs were found to have a significantly higher whole-organism metabolic rate than SWs (Crnokrak and Roff 2002; Nespolo et al. 2008), and functional DLMs have a higher metabolic rate than histolyzed or underdeveloped DLMs (Zera et al. 1997). However, none of the above studies have determined the specific relationship between the mass of the DLMs and increased allocation of nutrients to the soma versus reproductive tissues and/or an increased maintenance cost. An accurate proxy for allocation to flight capability must include the increased allocation to the soma and the possible increased maintenance cost of DLMs.

The primary goals of this experiment are: 1) to estimate total acquisition and total allocation (to flight capability and to reproduction) in female *Gryllus firmus*, and 2) to determine if energy acquisition and allocation can be accurately predicted from measurements of tissue masses alone. To achieve these objectives, we estimated both the energy content of body component tissues and the cost of developing and maintaining these tissues. We did this by measuring both the biochemical composition of different body tissues and the whole organism resting metabolic rate for individuals reared on two food levels at 7 days of age when the trade-off between these traits is the strongest (Crnokrak and Roff 2002). From these measurements, we estimated the total energy acquired by individuals and the total amount individuals allocate to flight capability and reproduction.

3.2 Methods

3.2.1 Rearing Conditions

The stock population for this experiment was created from 27 (likely multiply mated) females collected in September 2007 from Gainesville, FL (Lat: N 29.68°, Lon: W 82.27°; Figure 0.4). This stock was subsequently maintained at a size above 400 individuals to maintain levels of genetic diversity. We collected eggs from this stock population and once hatched, we apportioned nymphs among fifteen 1.8 Liter buckets at a density of 15–20 per cage. All cages were kept at 28°C and 50% relative humidity in environmentally controlled growth chambers (ECG; Chagrin Falls, Ohio). Nymphs were provided with ground LabDiet Prolab© Rabbit Chow ad libitum. We removed individuals from these cages on the day of their final ecdysis (final molt to the adult form). These adults were subsequently reared individually on one of two food treatments. The high food treatment consisted of ad libitum LabDiet Prolab© Rabbit Chow while the low food treatment consisted of a daily ration of LabDiet Prolab© Rabbit Chow previously determined to decrease reproductive output by 50% (King *unpublished data*). When adults were 7 days post-ecdysis, we measured each individual's metabolic rate (see Section 3.2.2 below). Individuals were then dissected, and DLMs and ovaries were removed, dried and weighed to the nearest 0.0001 g. The state of muscle histolysis was also recorded for each dissected cricket (2 = no evidence of histolysis, 1 = partially histolyzed, 0 = totally histolyzed or absent). These tissues were subsequently stored it at -20°C for future biochemical analysis.

3.2.2 Resting Metabolic Rate

The potentially increased maintenance cost of having large DLMs is an important issue when estimating the energy allocated to DLMs, because this allocation should include the energy devoted to the maintenance of these tissues. To estimate this cost, we measured the resting metabolic rate (hereafter, RMR) of 300 individuals 7 days of age at 28 ° C. Metabolic rate is known to increase immediately following feeding, and this effect is known as the heat increment of feeding (Chappell et al. 1997; Nespolo et al. 2005). Therefore, to prevent variation in metabolic rate associated with variation in the amount of food in the gut, individuals were fasted for at least 12 hours prior to measurement.

The respirometry apparatus used in this experiment was similar to that of Crnokrak and Roff (2002) and Nespolo et al. (2005). We used an open-flow system to measure the rate of CO_2 production ($\dot{V}CO_2$; mL CO_2/h) over a 25 minute period following a 5 minute acclimation period. Carbon dioxide production was measured using a infrared CO_2 analyzer capable of resolving differences of 1 part per million of CO_2 in air (Licor 6251; Lincoln, Nebraska). The analyzer was calibrated periodically against a precision gas mixture with a known CO_2 concentration. Dry, CO_2-free air was maintained at a flow rate of 190 mL/min by a mass flow controller (Sierra Instruments; Monterey, California). Activity levels were measured concurrently with CO_2 production using an activity detector (Sable Systems AD-1; Las Vegas, Nevada). The outputs from the CO_2 analyzer and activity detector were recorded on a Dell© PC equipped with a Sable Systems UI-2 A/D universal interface and Sable Systems Expedata software.

Carbon dioxide concentrations measured in parts per million were converted to fractional concentrations and $\dot{V}CO_2$ (mL CO_2/hr) was computed using the equation,

$$\dot{V}CO_2 = \frac{\dot{V}(FECO_2 - FICO_2)}{1 - FECO_2\left(1 - \frac{1}{RQ}\right)}$$

where $\dot{V}$ is the flow rate (mL/h) corrected to standard temperature and pressure, $FICO_2$ is the incurrent fractional concentration of CO_2 ($FICO_2$ = 0 in this experiment), $FECO_2$ is the excurrent fractional concentration of CO_2, and RQ is the respiratory quotient (ratio of CO_2 produced/O_2 consumed). We used an RQ of 0.85, which is the value generally used for herbivorous insects and has been used for *Gryllus firmus* in previous studies (Nespolo et al. 2005). In addition, RQ had a very small effect on calculated values of $\dot{V}CO_2$ because $FECO_2$ was very small in this study (<0.0005).

Resting metabolic rate was determined from each record by calculating the minimum continuous mean for a 10 minute period using the data analysis software LabAnalyst (Warthog Systems, http://warthog.ucr.edu). Any record that had an overall high level of activity or had a substantial activity signal within the 10 minute minimum period was eliminated. The final data set consisted of 137 LW and 37 SW individuals.

3.2.3 Biochemical Composition of Tissues

The energy content of entire crickets as well as of DLMs and ovaries was estimated from the total lipid, carbohydrate, and protein content of the tissues. Dorso-longitudinal muscles (DLMs) are composed of mostly protein but are known to also contain some carbohydrate in the form of glycogen (Gillott 1995). Ovaries contain mostly phospholipids and ovarian proteins, though yolks may also contain significant amounts of glycogen (Gillott 1995). For 44 LW females, we measured total lipid and carbohydrate content of ovaries, dorso-longitudinal muscles (DLMs) and remaining tissue following the methods of Van Handel (1985a, b) and Van Handel and Day (1988). Briefly, tissues were homogenized in a 2% $NaSO_4$ solution, which absorbs all glycogen. Soluble oligosaccharides (sugars) and lipids were then extracted with a chloroform/methanol mixture. The glycogen absorbed on $NaSO_4$ precipitates and was separated by centrifugation. One half the supernatant was used to quantify lipids while the other half was used to quantify sugars. Lipids were quantified photometrically using the vannilin reaction with 0.1% soybean oil in chloroform as a standard (Van Handel 1985b). Glycogen and sugars were quantified photometrically using the anthrone reaction with 0.1% glucose in 25% ethanol as a standard (Van Handel 1985a). Chitin co-precipitates with glycogen and interferes with the anthrone reaction. This interference was not an issue in the measurement of ovaries and DLMs, because neither contains any chitin. However, the remaining body tissue obviously contains significant amounts of chitin. Therefore, in these samples, chitin was separated from glycogen using a heated 30% KOH solution, and glycogen was then reprecipitated with ethanol, following methods developed by Van Handel (1965) and modified by Blanckenhorn (2007). After total lipid and carbohydrate were accounted for, the remaining mass was assumed to be protein. These components were then converted to units of energy (Joules) using standard values of energy density (Schmidt-Nielsen 1990).

The conversion above accounts for the energy content of these tissues, but these tissues have a synthesis cost. Several studies have shown that protein synthesis is less efficient than lipid synthesis in both higher vertebrates (Pullar and Webster 1977; Blaxter 1989; Birkett and de Lange 2001) and fish (Lupatsch et al. 2003). Therefore, tissues differing in the proportion of lipid and protein will have very different synthesis costs. Estimated efficiencies for protein and lipid deposition range from 44% to 60% (kJ deposited/kJ expended) for protein synthesis and 70% to 90% (kJ deposited/kJ expended) for lipid synthesis (Birkett and de Lange 2001) and do not differ substantially between endotherms and ectotherms (Lupatsch et al. 2003). Therefore, we used values from the center of the range of reported values: 52% for protein synthesis and 80% for lipid synthesis (i.e., total cost of 1 g protein is 42.7 kJ, or the sum of its energy content of 22.2 kJ plus its synthesis cost of 20.5 kJ). The theoretical efficiency of synthesizing carbohydrates calculated from biochemical considerations is 95% (Blaxter 1989), and this is the efficiency we used in this study. Carbohydrates do not contribute

Table 3.1: Mean (± SE) resting metabolic rate (mL CO_2/hr) and analysis of covariance of the effects of total dry mass, activity, wing morphology and food level on resting metabolic rate in *Gryllus firmus*.

Food	**Micropterous (SW)**		**Macropterous (LW)**	
Low	0.40 (0.0035)		0.44 (0.0009)	
High	0.45 (0.0055)		0.50 (0.0013)	
Source	**d.f.**	**SS**	***F***	***P***
Total Mass	1	0.2584	61.02	<0.001
Activity	1	0.0319	7.55	0.007
Wing Morph	1	0.0018	0.42	0.52
Food	1	0.0001	0.04	0.85
Wing Morph × Food	1	0.0055	1.30	0.26
Error	168	0.7113		

much to the energy content of the tissues measured and thus deviation from this theoretical efficiency will not have a great effect.

3.3 Results

3.3.1 Resting Metabolic Rate

Wing Morphology and Food

Average values of RMR (mL CO_2/hr) are shown in Table 3.1. These values are somewhat higher than predicted based on the expected scaling of metabolic rate with body size in insects (Chown et al. 2007), but they are within the range of previously reported values for Orthopterans (Reinhold 1999; Chown et al. 2007).

The effect of wing morphology and food level on metabolic rate was determined using an ANCOVA. Metabolic rate is expected to scale with body size and will also be influenced by activity level and therefore, both total body mass (dry mass) and activity were included as covariates. While records with high activity levels were eliminated, we included activity in the

Table 3.2: Mean (± SE) total dry mass (g) and analysis of variance of the effects of wing morphology and food level on total dry mass in *Gryllus firmus*.

Food	**Micropterous (SW)**		**Macropterous (LW)**	
Low	0.20 (0.0017)		0.22 (0.0004)	
High	0.25 (0.0020)		0.29 (0.0007)	
Source	**d.f.**	**SS**	***F***	***P***
Wing Morph	1	0.026	18.0	<0.001
Food	1	0.089	62.7	<0.001
Wing Morph × Food	1	0.001	1.01	0.32
Error	170	0.242		

analysis to account for any variation in metabolic rate due to small activity differences. Activity was significant but explained a very small proportion of the variance in metabolic rate (Semi-partial R^2 = 4%). Neither wing morph, food level, nor the interaction was significant once total body mass and activity were taken into account (Table 3.1). An ANOVA shows that food level and wing morph significantly affect total body mass (Table 3.2). Macropterous (LW) individuals are larger than SW individuals and the lower food level produced smaller crickets. Thus, while LWs have a higher raw RMR than SWs and individuals on high food have a higher raw RMR than those on low food, these differences reflect differences in body size. Once body size was accounted for, RMR was not significantly different between these groups.

Body Components and RMR

To determine if the body components DLM mass, ovary mass, and remaining body tissue mass influence metabolic rate disproportionately, we compared two linear regression models using Akaike's Information Criterion (Table 3.3). One model predicted RMR including the body components separately while the other included only total body mass. If the body components influence metabolic rate disproportionately, the model including the body components separately that allows for the estimation of different regression coefficients will perform better. Because previous work suggests larger DLM masses may be associated with a greater

Table 3.3: Regression coefficients and AIC values for two models predicting resting metabolic rate. Statistically significant coefficients are bold.

Model	**Coefficient**	**AIC**
Total Body Mass		-329.2
Total Body Mass	**0.98**	
Activity	2.83	
Body Component Masses		-326.4
DLM Mass	3.48	
Ovary Mass	**1.30**	
Remaining Body Mass	**0.81**	
Activity	2.21	

maintenance cost (Zera et al. 1997; Crnokrak and Roff 2002; Nespolo et al. 2008), for this analysis we included only individuals that had not histolyzed their DLMs. Food treatment was not included in these models due to its lack of statistical significance in the analysis above. When comparing AIC values, the lowest value indicates the best model (Burnham and Anderson 1998), which in this case was the model including only total mass (Table 3.3). This result indicates that total mass is sufficient to explain variation in metabolic rate and that the body components are not influencing metabolic rate disproportionately. The lack of a statistically significant relationship between DLM mass and metabolic rate was confirmed by an insignificant regression between residual metabolic rate (i.e., the residuals from the regression of RMR on total mass and activity) and DLM mass (Figure 3.1).

3.3.2 Biochemical Composition of Tissues

As expected, the amount of lipids, sugars and glycogen significantly increased with increasing tissue masses for DLMs, ovaries and remaining body tissues (Figure 3.2). Total carbohydrates (glycogen and sugars) did not contribute much to overall energy content accounting for on average: 1.05% for DLMs, 1.58% for ovaries and 0.23% for remaining tissue. Lipids accounted for a greater proportion of overall energy content (23.3% for DLMs, 16.8% for

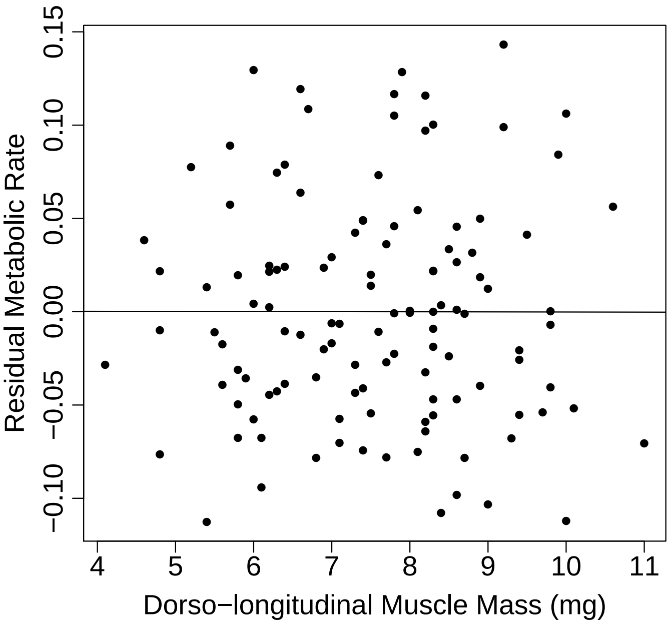

Figure 3.1: Residual metabolic rate as a function of dorso-longitudinal muscle mass (mg). Residual metabolic rate values are the residuals from a regression of resting metabolic rate on total body mass and activity.

ovaries and 9.03% for remaining body tissue). Protein contributed the most to energy content (75.6% for DLMs, 81.6% for ovaries, and 90.7% for remaining tissue).

Food Level and Mass

The total energy devoted to each body component was calculated including the cost of synthesizing the tissues (hereafter simply total energy). We used an ANCOVA to test the effects of tissue mass, food level and the interaction on total energy for each component. A significant interaction would indicate that individuals were shifting the composition of their tissues depending on food level. We did not find evidence for a food by mass interaction for DLMs ($F_{1,40} = 0.07$, $P = 0.78$), ovaries ($F_{1,40} = 2.2$, $P = 0.15$), or remaining body tissue ($F_{1,40} = 0.09$, $P = 0.76$), while mass was always strongly significant (DLMs: $F_{1,40} = 240620$, $P < 0.001$; Ovaries: $F_{1,40} = 16459$, $P < 0.001$; Remaining Body Tissue: $F_{1,40} = 308070$, $P < 0.001$). Food

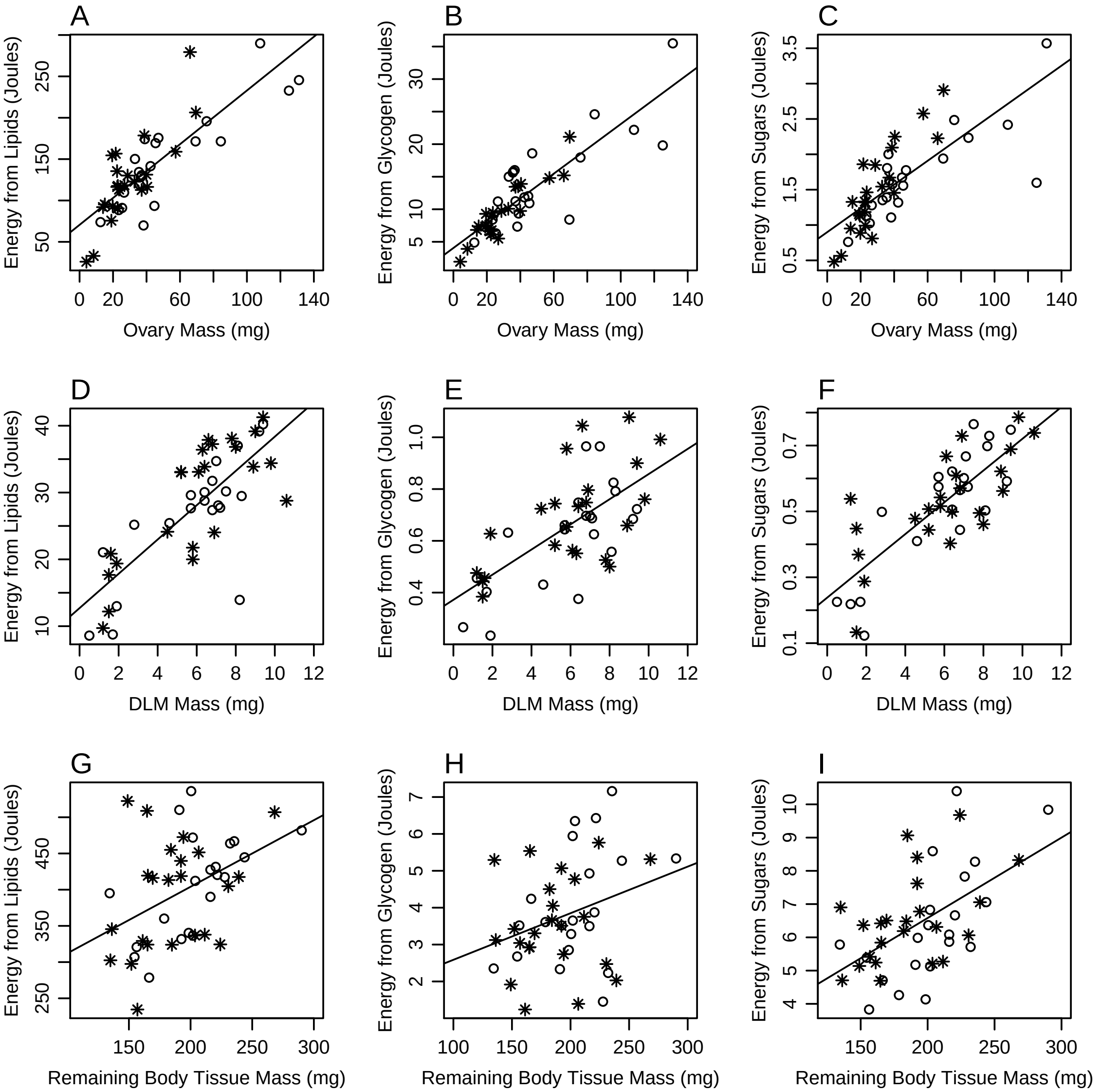

Figure 3.2: A–C) The relationship between ovary mass (mg) and A) energy content from lipids (Joules), B) energy content from glycogen (Joules), and C) energy content from sugars (Joules). D-F) The relationship between DLM mass (mg) and D) energy content from lipids (Joules), E) energy content fom glycogen (Joules) and F) energy content from sugars (Joules). G-I) The relationship between remaining body tissue (minus ovaries and DLMs) mass (mg) and G) energy content from lipids (Joules), H) energy content from glycogen (Joules), and I) energy content from sugars (Joules). ○ = High Food, $*$ = Low Food.

level was not significant beyond its effect on mass (DLMs: $F_{1,40}$ = 0.009, P = 0.92; Ovaries: $F_{1,40}$ = 2.69, P = 0.11; Remaining Body Tissue: $F_{1,40}$ = 0.095, P = 0.77).

3.3.3 Predictive Model

We used the estimates of RMR and of the biochemical composition of body components to construct a model predicting total energy acquisition and total energy allocated to reproduction and flight capability from tissue masses. These models included the energy content and synthesis costs calculated from the lipid, carbohydrate, and protein content as described above, as well as the energy devoted to the maintenance of the tissue. Because we found that RMR was not influenced disproportionately by any body component, the maintenance cost for each component was calculated by multiplying the whole-organism RMR by the fraction of body mass consisting of that component. We then converted ml CO_2/min to Joules/min by assuming an equivalence of 24.65 Joules/mL CO_2 (Chown et al. 2007) and extrapolated across the first 7 days of adulthood. Details for each estimate are below.

Total Acquisition

Total acquisition includes the total energy content and synthesis cost of entire individuals, as well as the whole-organism maintenance costs. To incorporate whole-organism maintenance costs, we assumed that individuals eclosed as adults at 80% of their final mass, which corresponds to the average increase in total body mass over the first week of adulthood (Zera and Larsen 2001). The energy devoted to maintenance was calculated from the above RMR data accounting for changes in mass from the final ecdysis until an age of 7 days. To determine how total acquisition can be predicted from total mass, we regressed total body mass against total acquisition. Total acquisition can be predicted very well from total body mass (R^2 = 0.99, P < 0.001; Figure 3.3A).

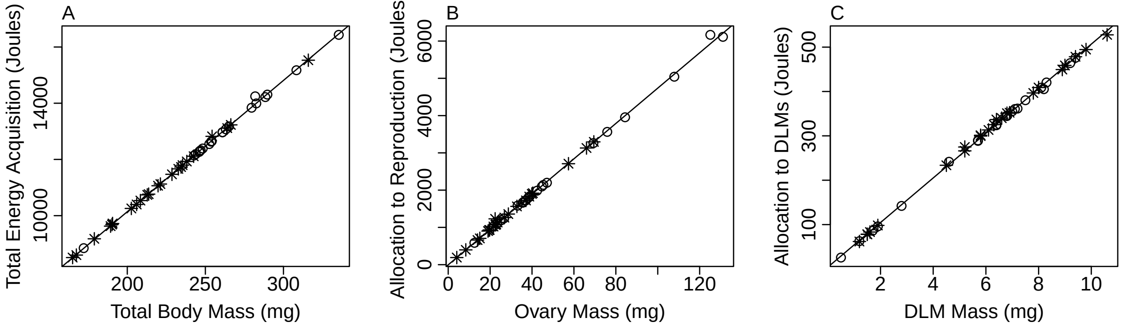

Figure 3.3: A) Total acquisition (J) vs. Total body mass (mg), B) Allocation to reproduction (J) vs. Ovary mass (mg), C) Allocation to dorso-longitudinal muscles (J) vs. Dorso-longitudinal muscle mass (mg), ○ = High Food, ∗ = Low Food.

Allocation to Reproduction

Allocation to reproduction includes the total energy content and synthesis cost of ovaries, as well as the cost of maintaining ovaries. To estimate the cost of maintaining ovaries, we assumed individuals did not have measureable ovaries at the final ecdysis and that ovaries grew at a constant rate across the first week of adulthood. Once again, energy devoted to maintenance of ovaries was calculated from the above RMR data accounting for changes in mass from the final ecdysis until an age of 7 days. Energy allocated to reproduction is predicted very well from ovary mass ($R^2 = 0.99$, $P < 0.001$; Figure 3.3B). According to this model, individuals allocate 47 Joules for each milligram of ovary tissue.

Allocation to Flight Capability

The energy allocated to DLMs was calculated including the total energy content and synthesis cost of DLMs, as well as the cost of maintaining DLMs. At the final ecdysis, LWs have fully developed DLMs (Zera et al. 1997; Roff and Fairbairn *unpublished data* for this population). Therefore, we did not assume any change in mass across the first week of adulthood. Not assuming a decline in mass for individuals that have partially or totally histolyzed their

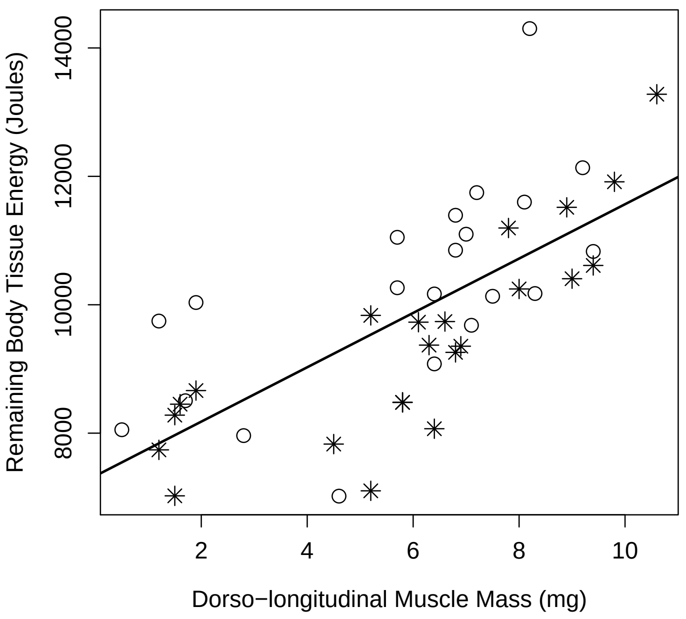

Figure 3.4: Energy allocated to remaining body tissue (Joules) as a function of dorso-longitudinal muscle mass (mg). ∘ = High Food, ∗ = Low Food.

flight muscles may underestimate the maintenance cost for some individuals with smaller or no muscles. However, DLMs are fairly small (~5% of total mass), therefore underestimation of the maintenance cost will have a relatively small effect. The energy allocated to DLMs is predicted very well from DLM mass ($R^2 = 0.99$, $P < 0.001$; Figure 3.3C).

However, allocation to flight capability involves a suite of traits in addition to the energy allocated to the DLMs (see Introduction and references therein). Therefore, to determine the relationship between DLM mass and the amount of energy stored in the soma, we used least squares regression. DLM mass explained 50% of the variation in the amount of energy allocated to remaining body tissue ($R^2 = 0.50$, $P < 0.001$; Figure 3.4). This effect is not simply a result of individuals with larger DLMs having a large total body mass. The correlation between DLM mass and the remaining body mass (soma) is high and significant while that between total body mass and DLM mass is lower and non-significant (Table 3.4). We then assume the energy allocated to the remaining tissue mass that is accounted for by DLM mass is energy

Table 3.4: Pearson correlation coefficients between body components. Statistically significant coefficients are bold.

	DLM Mass	Ovary Mass	Remaining Body Mass	Total Mass
DLM Mass	*	**-0.64**	**0.71**	0.24
Ovary Mass		*	-0.27	**0.44**
Remaining Body Mass			*	**0.75**

Note: Remaining body mass is equal to the mass of the tissues excluding ovary mass and DLM mass.

stored for flight. Accounting for the energy allocated to DLMs and this additional allocation of energy to the soma, each milligram of DLM tissue corresponds to an allocation of 473 J to flight capability.

3.4 Discussion

Differential allocation of nutrients has been extensively studied in different wing morphs of *G. firmus* (Zera et al. 1994; Zera and Denno 1997; Zera and Brink 2000; Zera and Larson 2001; Crnokrak and Roff 2002; Zera 2005; Zera and Zhao 2006). However, the trade-off between flight capability and reproduction exists not just at the level of the two wing morphs but within flight capable morphs as well (Fairbairn and Roff 1990; Roff 1994). So, while it has been well established that wing morphs differentially allocate their resources toward flight capability and reproduction, this study is the first to explicitly elucidate the relationship between variation in energy allocation and variation in tissue masses.

With respect to allocation of energy to maintenance, we found no evidence for an increased maintenance cost associated with larger DLMs. Different tissues have different contributions to overall metabolic rate. For example, in vertebrates, organs such as the kidneys and the brain contribute disproportionately to overall resting metabolic rate (Itazawa and Oikawa

1986). It has been suggested that maintaining large flight muscles (DLMs) will lead to a higher maintenance cost (Zera et al. 1997; Crnokrak and Roff 2002; Nespolo et al. 2008). In this study, we found the main determinant of resting metabolic rate was total body mass alone and that DLM mass did not contribute disproportionately to overall metabolic rate. This result contrasts with a previous study by Crnokrak and Roff (2002) showing an increased whole-organism metabolic rate in LW females with functional DLMs compared to SW morphs. This difference may result from differences in the way metabolic rate was measured. The previous study did not examine the relationship between DLM mass and overall metabolic rate, and, in this study, we did not find evidence for an additional cost of maintaining large DLMs above that required to maintain other body tissues.

Flight muscle (DLM) mass was associated with increased allocation of energy to the soma. Individuals with larger DLMs had a larger energy reserve in their remaining body mass. This result is consistent with previous studies showing LWs have higher somatic levels of protein and lipid (Zera 2005; Zhao and Zera 2006). Triglycerides are the main flight fuel for Orthopterans and therefore building up a store of triglycerides is critical for flight capability (Gillott 1995). Previous studies also show that LWs preferentially oxidize amino acids as opposed to fatty acids to fuel production of triglycerides (Zhao and Zera 2006). Therefore, this increased allocation to the soma associated with increasing DLM mass should be included in allocation to flight capability.

With this new information, we can construct a predictive model of energy acquisition and energy allocation from tissue masses at the point when the trade-off is the strongest, at 7 days of age (Crnokrak and Roff 2002). Our estimate of total energy acquisition includes the total energy content in individual crickets in addition to the energy required to grow and maintain those tissues and can be predicted very well from total body mass. Energy allocation to reproduction is relatively straightforward in this species. Virgin females retain and do not reabsorb their eggs and total fecundity is correlated with ovary mass (Roff 1994). Therefore, the energy allocated to reproduction can be estimated as the total energy content of ovaries plus the cost of synthesizing and maintaining that tissue. Allocation to flight capability is more complicated and involves not only allocation to DLMs but allocation to energy stores as well. Therefore, the energy allocated to flight capability includes the energy content, synthesis and maintenance costs of DLMs and the additional energy stored in the soma that scales with DLM mass. These estimates for allocation reflect a more accurate representation of relative allocation to ovaries and flight muscles than the masses of these organs alone.

Estimating total energy acquisition and energy allocation to various functions presents a significant challenge to researchers. However, trade-off patterns are expected to be related to the relative variances in energy acquisition and energy allocation (van Noordwijk and de Jong 1986; Reznick et al. 2000), and, therefore, estimating these quantities is critical for studies of the energetic basis of trade-offs. In this study, we were able to demonstrate that total energy acquisition, allocation to reproduction, and allocation to flight capability can be predicted from tissue masses alone in *G. firmus*. The ability to estimate relative acquisition and allocation from simple mass measurements will significantly simplify future studies where detailed biochemical measurements are not feasible in this model system.

3.5 References

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Chapter 4

The evolutionary genetics of phenotypic plasticity in acquisition and allocation in the wing dimorphic cricket, *Gryllus firmus*

4.1 Introduction

Genetic and phenotypic variation in the acquisition of resources and the subsequent allocation of those resources to different traits can profoundly affect the correlation between traits involved in a functional trade-off (James 1974; Riska 1986; van Noordwijk and de Jong 1986; Houle 1991). Van Noordwijk and de Jong (1986) developed a model, called the "Y model", demonstrating how variation in acquisition and allocation will influence the relationship between traits in a trade-off. Their model consists of two traits drawing from a common resource pool and includes variation in both the size of the total resource pool (acquisition) and the proportion of resources allocated to each trait (allocation). For a fixed acquisition, variation in allocation leads to a negative covariance between traits, as we typically expect for traits involved in a trade-off. If, however, acquisition also varies, and some individuals in a population are able to acquire more resources than others, those individuals will have a larger resource pool and can allocate more resources to both traits. This effect can lead to a positive correlation between these traits when measured across individuals in a population even while there is a resource-based trade-off within individuals. Specifically, the Y model predicts that the sign of the correlation will depend on the relative variation in acquisition and the relative variation in allocation. The basic Y model of van Noordwijk and de Jong (1986) has been extended to explicitly include genetic and environmental variation (de Jong and van Noordwijk 1992), several loci (Houle 1991), several traits (de Laguerie et al. 1991; de Jong 1993; Worley 2003), and phenotypic plasticity (Malausa et al. 2005). However, all of these models maintain the basic principle that trade-offs depend critically upon the relative variation in acquisition versus allocation. Thus, any genetic and phenotypic variation in the acquisition of resources and subsequent allocation of those resources will influence the expression of trade-offs in natural populations.

Given that variation in acquisition relative to variation in allocation is predicted to govern trade-off patterns, to understand how and why trade-offs vary in natural populations, we must study the sources of that variation. Substantial genetic variation may exist for the acquisition and/or allocation of resources. These traits may also be phenotypically plastic in the sense that the phenotypic value of a genotype changes in response to the environment. Phenotypic variation is not exclusive of genetic variation; genetic variation for a plastic response can and indeed must exist for phenotypic plasticity to evolve. This variation in a plastic response among genotypes is referred to as a genotype by environment interaction (Pigliucci 2001). These three sources of variation—genotype, environment, and genotype by environment interaction (hereafter G×E)—are of critical importance in determining trade-off patterns and are the focus of this study.

Environmentally induced variation in acquisition caused by spatial and temporal variation in resource levels is certainly common in natural populations, and variation in acquisition is commonly assumed to result exclusively from this source of variation (e.g., Glazier 1999; Bashey 2006). However considerable genetic variation in acquisition may also be present in many populations. Individuals may differ in traits such as feeding rate, digestive efficiency, or gut capacity, any of which could lead to genetically based differences in acquisition (Ricklefs 1991; Reznick et al. 2000).

Empirical evidence also exists for a genotype by environment interaction between acquisition in low versus high resource environments. If no single genotype has the highest acquisition in all resource environments, a genotype by environment interaction exists, which could act to maintain genetic variation in acquisition (Gillespie and Turelli 1989). Several studies have found that individuals can increase their acquisition efficiency in resource-stressed environments, and the observation that these shifts only occur under resource stress suggests that they incur some cost under normal conditions (Ricklefs 1991; Weiner 1992; Hammond and Diamond 1997). Studies of populations adapted to different resource environments also show evidence for a genotype by environment interaction. Both *Drosophila* (Leroi et al. 1994) and *Daphnia* (Tessier and Woodruff 2002) populations from low resource environments have been shown to acquire more resources than those from a high resource environment under low resource conditions, and the opposite is true under high resource conditions. Despite these findings, few studies of the genetic variation for resource acquisition exist.

In contrast, allocation is often studied from the perspective of quantitative genetics and most studies of trade-offs can be viewed as studies of the quantitative genetics of differential resource allocation (e.g., Geber 1990; Stirling et al. 1999; Messina and Fry 2003). However, trade-offs are most commonly studied in a single environment, or at most in two different environments. Theory suggests that genetic parameters can shift substantially in different environments due to genotype by environment interactions, and thus the expression of genetic variance may shift with resource level (Stearns et al. 1991; Hoffmann and Merila 1999; Charmantier and Garant 2005). Empirical evidence also shows that allocation patterns often vary with variation in resource level (Bell and Koufopanou 1985; Sgrò and Hoffmann 2004). Phenotypic plasticity in allocation patterns in response to variation in resource levels has been found in a wide variety of organisms including insects (Gebhardt and Stearns 1988; Kaitala 1991; Chippindale et al. 1993; Simmons and Bradley 1997; Blanckenhorn 1998; Czesak and Fox 2003; Messina and Fry 2003), reptiles (Jordan and Snell 2002), fish (Bashey 2006), mammals (Ruf et al. 2006), cladocerans (Yampolsky and Ebert 1994; Tessier et al. 2000), bivalves (Jokela and Mutikainen 1995; Ernande et al. 2004), and plants (Biere 1995). However, few studies have examined the evolutionary potential of phenotypic plasticity in allocation in multiple environments.

In this study, we estimated the genetic architecture of acquisition and allocation in a model system for the study of trade-offs, the wing dimorphic cricket, *Gryllus firmus*. We performed a large-scale quantitative genetic breeding experiment, in which half-sibling families were split over several resource levels with the aim of addressing the following questions:

1. What is the pattern of plasticity in allocation in response to resource level?
2. What is the evolutionary potential of phenotypic plasticity in allocation?
3. What is the evolutionary potential of acquisition and allocation?
4. Do genetic parameters change in different resource environments?
5. Is there a genotype by environment interaction for acquisition?

4.2 Methods

4.2.1 Study system

Gryllus firmus is one of the most well studied wing dimorphic insect species and has emerged as a model system for the study of trade-offs. Like other wing dimorphic species, *G. firmus* trade off reproductive output with flight capability (for reviews see Harrison 1980; Roff 1986; Dingle 1996; Zera and Denno 1997). Flight capable morphs are macropterous with long wings (hereafter LW), while flightless morphs are micropterous with short, non-functional wings (hereafter SW). Even in the absence of flight, making and maintaining the large flight muscles incurs a significant energetic cost (Harrison 1980; Roff 1984; Roff 1986a; Dingle 1996; Zera and Denno 1997; Zera et al. 1997; Crnokrak and Roff 2002). Macropterous individuals have significantly larger dorso-longitudinal muscles (hereafter DLMs), the main flight muscles, than micropterous individuals. In contrast, micropterous individuals begin reproducing earlier and have a higher cumulative fecundity (Roff 1984, 1994; Zera et al. 1997; Roff and Gelinas 2003). In addition, LW females also allocate more protein and lipid to the soma, while SW females allocate more protein and lipid to ovaries (Zera and Zhao 2006). This trade-off also exists within macropterous morphs, because individuals with larger DLMs have, on average, smaller ovaries (Roff et al. 2002; Roff and Gelinas 2003). Many physiological studies have also demonstrated differences in the allocation of available nutrients in flight capable compared to flightless morphs (Zera et al. 1994; Zera and Denno 1997; Zera and Brink 2000; Zera 2005; Zhao and Zera 2006), providing a strong argument that these two traits are in a functional trade-off and compete for resources.

In crickets, the switch determining whether an individual will be LW or SW can occur as late as the last nymphal instar (Zera and Denno 1997). At the final ecdysis, LW *G. firmus* individuals have fully developed DLMs (Zera and Mole 1994; Zera et al. 1997; Roff and Fairbairn *unpublished data for this population*). Most LW individuals histolyze their flight muscles within the first two weeks following eclosion into the adult form (Fairbairn and Roff 1990; Stirling et al. 2001), and fecundity following histolysis increases dramatically by allowing the resources otherwise devoted to flight muscle to be reallocated to egg production (Roff 1989). Micropterous (SW) individuals have very small, non-functional DLMs at eclosion (Zera and Mole 1994). Ovary masses are similar between the two morphs at eclosion but by day seven, SW females have significantly larger ovaries than LW females (Zera and Brink 2000; Crnokrak and Roff 2002).

4.2.2 Rearing Protocol

The experimental design consisted of a split-plot, half-sibling rearing experiment in which each male (sire) was mated with three females (dams), and the offspring of each dam reared in three different diet treatments. Parents were chosen at random from a stock population started from 27 females (assumed to be multiply mated) collected in September 2007 from Gainesville, FL (Lat.: N 29.68°, Lon.: W 82.27°). Females were assigned a unique number, placed in their own mating container, and provided with a moist cotton pad as an oviposition site. Each male was rotated among three females every two days to create half-sibling families. After hatching, nymphs from each dam were split between two 1.8 Liter buckets to control for microenvironmental effects. Nymphs were provided with ground LabDiet Prolab© Rabbit Chow *ad libitum*. On the day of their final ecdysis (final molt to the adult form), individuals were placed in individual 0.5 Liter cages and assigned to one of three food treatments. The high food treatment consisted of *ad libitum* LabDiet Prolab© Rabbit Diet, the 50% diet consisted of a daily ration of food determined to reduce ovary mass by 50% (E. King *unpublished data*), and the low food treatment consisted of a daily ration of food just above the amount required for survival for 20 days (Crnokrak and Roff 1998). All cages were kept at 28° C and 50% relative humidity in environmentally controlled growth chambers (ECG; Chagrin Falls, Ohio). Up to ten females from each half-sib family in each food treatment were preserved in 70% ethanol at seven days of age (past the final ecdysis) for subsequent dissection and measurement, although mortality occasionally prevented the collection of the full ten individuals (mean = 9.1, sd = 1.5). The final data set consisted of 5,080 offspring from 63 sires and 186 dams.

Each cricket was dissected, dorso-longitudinal muscles (DLMs) and ovaries were removed, dried, and weighed to the nearest 0.0001 g. We also measured the mass of the remaining body mass after both ovaries and flight muscles were removed (hereafter remaining mass). The state of muscle histolysis was also scored for each dissected cricket using a three-level scale (no evidence of histolysis, partially histolyzed, and totally histolyzed or absent).

4.2.3 Acquisition and Allocation Estimation

For most life history trade-offs, it is assumed that energy is the major limiting resource and in this case, total acquisition will be the total energy acquired by an organism and allocation will be the proportion of that energy allocated to various traits. The amount of energy allocated to reproduction was predicted from ovary mass according to the model developed in Chapter 3, incorporating energy content, synthesis costs, and maintenance costs. Similarly, the amount of energy allocated to flight capability was predicted from DLM mass according to the model developed in Chapter 3 incorporating energy content, synthesis costs, maintenance costs, and the energy stored for flight. Total energy acquisition was predicted from total body mass also according to the model developed in Chapter 3, incorporating energy content, synthesis costs and maintenance costs. From these quantities, acquisition and allocation can be defined in two ways. First, we calculate allocation to flight capability and to reproduction as proportions of the total energy budget (total acquisition). Hereafter these are called simply flight allocation and reproductive allocation.

Alternatively, in the Y model, only a single trade-off relationship is considered, and acquisition and allocation refer only to the focal trade-off. Therefore, in this model the appropriate total resource pool is actually the total resource pool available only for that trade-off. Thus, acquisition can also be estimated as the total energy allocated to reproduction + the total energy allocated to flight capability (hereafter trade-off acquisition). When acquisition is estimated this way, allocation is the proportion of this smaller energy budget (trade-off acquisition) allocated to flight capability or reproduction. Because only two functions are used, in this case, we only require one measure of allocation. The proportion allocated to one function will be one minus the proportion allocated to the other function. The choice between allocation to flight capability or to reproduction is arbitrary and herein, we use allocation to flight capability (hereafter trade-off allocation).

4.2.4 Statistical Analysis

We analyzed these data using the animal model approach described in Kruuk (2004) with ASReml (ver. 3.0; VSN International Ltd., Hemel Hempstead, UK). The animal model approach uses a mixed model restricted maximum likelihood (REML) procedure in which the unit analyzed is the individual, and a pedigree is used to define familial relationships. This model has the advantage of being able to handle unbalanced data sets. We transformed most trait values to improve normality, though REML procedures are fairly robust to deviations from normality (Kruuk 2004). Dorso-longitudinal muscle mass, flight allocation, and trade-off allocation were squared while ovary mass and reproductive allocation were log-transformed. Using this model, variance components, such as phenotypic variance and additive genetic variance can be estimated for any random effect, and genetic parameters can then be estimated from the calculated variance components. For a detailed description see Lynch and Walsh (1998), Kruuk (2004), and Wilson et al. (2010). Except where noted, all *P*-values were adjusted for multiple comparisons using the false discovery procedure described by Benjamini and Hochberg (1995).

To determine the overall pattern of plasticity, the effects of wing morphology and food treatment on the mean trait values can be tested by including wing morphology, food treatment, and their interaction as fixed effects in an animal model. The significance of these effects is given by a conditional Wald *F*-test (Wilson et al. 2010).

In all subsequent analyses in which we estimate genetic parameters, we included only LW females. Our study focuses on the change in the trade-off between flight capability and reproduction across food levels. Micropterous females (SW) have already made the allocation ‘decision’ to be flight incapable and will universally allocate little to flight capability (see Study System and references therein). Therefore, including these individuals in the analysis will only serve to obscure the response shown in LW females. Additionally, our data set consists of 88% LW females, thus excluding SW females did not substantially decrease our sample size. These results do not differ substantially from an analysis in which wing morph is included as a fixed effect (Appendix A on page 178).

We tested for maternal effects by comparing the likelihood of the model including dam as a random effect with the likelihood of the model without dam using a likelihood ratio test. This test follows a distribution with one degree of freedom, and the test statistic is equal to twice the difference in log likelihoods. We found no evidence for significant maternal effects (data not shown). We tested for cage effects in the same way and found no significant cage effect (data not shown). Therefore, in the following analyses, both dam and cage were excluded from the model.

4.2.5 Genetic correlations between environments

Phenotypic plasticity can be studied in a quantitative genetic sense by treating the trait expressed in each environment as a separate trait and calculating a genetic correlation between environments. To estimate the pairwise genetic correlations between environments (food treatments), we used a bivariate animal model where the trait expressed in each environment was considered a separate trait (Wilson et al. 2010). Because each individual is only measured in a single environment, missing values are assigned for the trait in the second environment (see http://www.wildanimalmodels.org). We estimated genetic correlations across environments for each pair of food treatments for each trait. The genetic correlation between any given pair of environments (a, b) is calculated as:

$$\frac{COV_{a,b}}{\sqrt{V_a V_b}}$$

A genetic correlation significantly less than one indicates a significant genotype by environment interaction. We tested whether our estimated genetic correlations were significantly different from one by comparing the full model with a model in which the correlation between the environments is constrained to be one with a likelihood ratio test (http://www.wildanimalmodels.org/). In ASReml, the correlation is set to be 0.9999 (if the correlation were set to be equal to one, the model would assume equal variances in the two environments, which will not necessarily be the case). In this case, the likelihood ratio test is a one-tailed χ^2 test, because the genetic correlation is bounded at one.

4.2.6 Heritabilities and genetic correlations between traits

Additive genetic variance, phenotypic variance and narrow sense heritabilities were estimated separately for each food treatment with wing morphology included as a fixed effect.

The narrow sense heritability was estimated from the additive genetic variance and phenotypic variance as:

$$\frac{V_A}{V_P}$$

The significance of the additive genetic variance was determined from a likelihood ratio test comparing the model including the additive genetic effect with the model excluding it as described above.

Genetic correlations between traits were calculated pairwise within each food treatment using a multivariate animal model with wing morphology included as a fixed effect. For any two traits (1, 2), the genetic correlation is calculated as:

$$\frac{COV_{1,2}}{\sqrt{V_1 V_2}}$$

The significance of the genetic correlation is determined by comparing the full model with a model in which the covariance between the traits is constrained to be zero ($COV_{1,2} = 0$) with a likelihood ratio test.

4.2.7 Changes in genetic architecture across environments

To test whether the estimated genetic parameters differed in the different food treatments, we used a delete-one jackknife procedure followed by a MANOVA. The delete-one jackknife is a re-sampling technique that has been shown to perform well for estimating both heritability (Knapp et al. 1989; Simons and Roff 1994) and genetic correlations (Roff and Preziosi 1994). This method followed by a MANOVA has been used to compare G matrices in previous studies (Roff 2002a; Bégin et al. 2004). In the delete-one jackknife, one sire family is deleted and a "pseudovalue" of each genetic parameter is calculated. This process is repeated, eliminating each sire family once. This procedure produces a set of pseudovalues for each genetic parameter in each food treatment. We then tested for differences between the food treatments using a MANOVA with the set of genetic parameters as the response variables and food treatment as the explanatory variable.

4.3 Results

4.3.1 Pattern of Phenotypic Plasticity

Wing morphology, food treatment, and their interaction significantly influenced all traits (Table 4.1). Mean trait values for different wing morphs and food treatments are given in Table 4.2 and Figure 4.1. The effect of wing morphology on trait values was consistent with previous findings (see Section 4.2.1 Study System and references therein). Micropters (SW) had larger ovaries, smaller DLMs, and smaller remaining mass (the mass minus DLMs and ovaries) than macropters (LW). Allocation to flight and reproduction followed these patterns with SWs allocating very little to flight. Acquisition, both total acquisition and trade-off acquisition was lower in SW females.

Food treatment also influenced trait values predictably. As expected, all masses increased with increasing food level, as did total acquisition and trade-off acquisition. Reproductive allocation increased with increasing food level for both morphs. Wing morphs differed in the response of flight allocation to increasing food levels. Increasing food levels lead to a slight decline in flight allocation in LW females, while it remained constant and very low for SW females. Trade-off allocation showed a stronger decrease with increasing food level for LW females but was again constant and low for SW females. This difference between wing morphs in the response to food level is the most obvious wing morph by food interaction (Figure 4.1).

Table 4.1: Conditional Wald F-tests for the effects of wing morphology, food treatment, and food by wing morphology interaction.

Trait	**df$_{\text{num,denom}}$**	$\boldsymbol{F}_{1,2}$	**Adjusted *P***
DLM Mass			
Food	1,5062	945.7	<0.001
Wing Morph	2,4889	263.6	<0.001
Food×Wing Morph	2,4936	37.0	<0.001
Ovary Mass			
Food	1,5067	525.5	<0.001
Wing Morph	2,4894	1650	<0.001
Food×Wing Morph	2,4943	20.0	<0.001
Remaining Mass			
Food	1,5034	861.0	<0.001
Wing Morph	2,4887	1403	<0.001
Food×Wing Morph	2,4922	19.7	<0.001
Total Acquisition			
Food	1,5016	104.1	<0.001
Wing Morph	2,4888	4474	<0.001
Food×Wing Morph	2,4917	4287	0.014
Flight Allocation			
Food	1,5072	1155	<0.001
Wing Morph	2,4897	94.2	<0.001
Food×Wing Morph	2,4950	9.6	<0.001
Reproductive Allocation			
Food	1,5070	711.2	<0.001
Wing Morph	2,4894	967.7	<0.001
Food×Wing Morph	2,4945	25.7	<0.001
Trade-off Acquisition			
Food	1,5060	114.4	<0.001
Wing Morph	2,4889	3098	<0.001
Food×Wing Morph	2,4935	24.2	<0.001
Trade-off Allocation			
Food	1,5072	1276	<0.001
Wing Morph	2,4896	451.1	<0.001
Food×Wing Morph	2,4949	52.5	<0.001

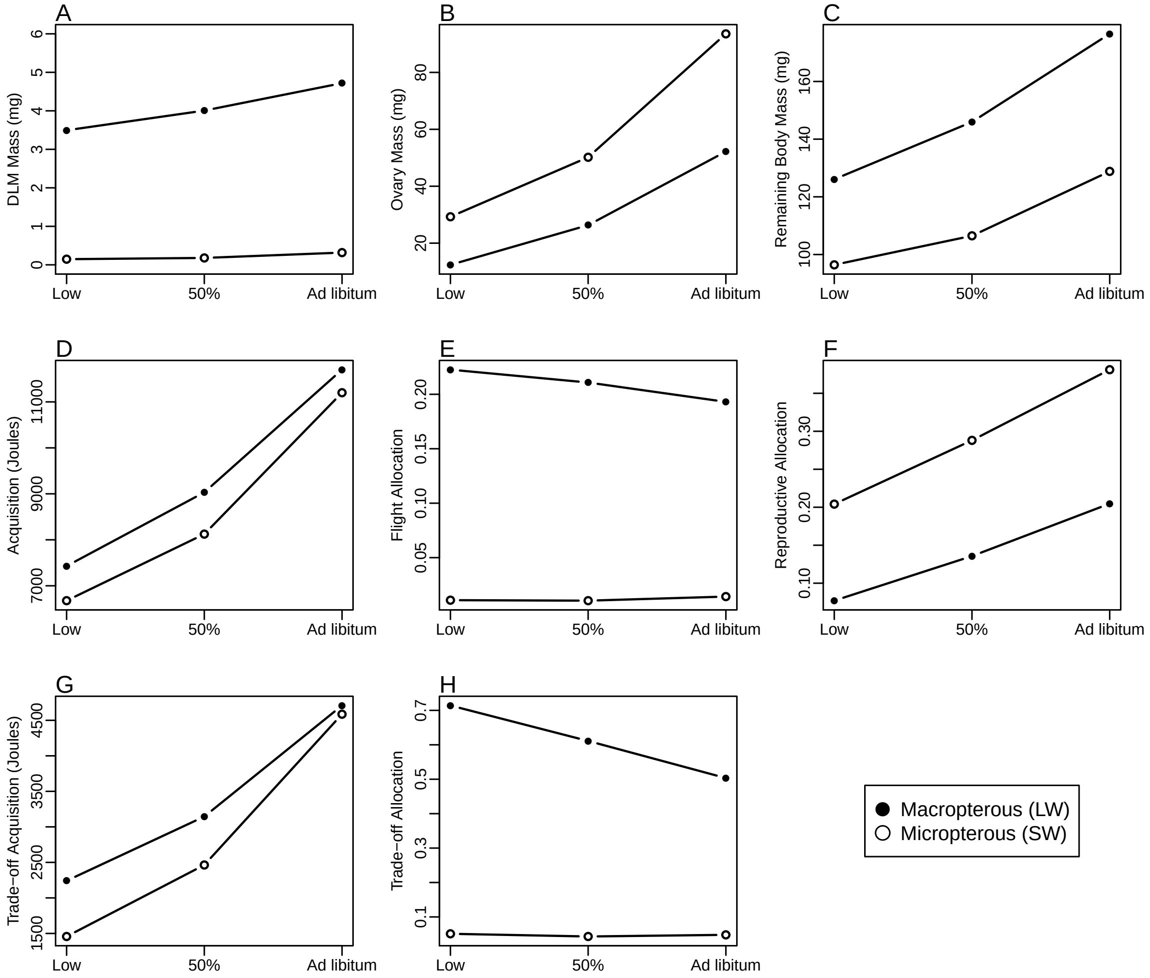

Figure 4.1: Mean trait values by wing morphology and food treatment. Standard errors are smaller than the height of the points and are not shown.

Table 4.2: Means and standard errors for each trait by food treatment and wing morphology.

Traits	Low Food	50% Food	*Ad libitum*
Macropters (LW)			
DLM Mass (mg)	3.5 (0.001)	4.0 (0.001)	4.7 (0.002)
Ovary Mass (mg)	12.4 (0.009)	26.3 (0.015)	52.2 (0.025)
Remaining Mass (mg)	126.1 (0.015)	146.0 (0.018)	176.5 (0.025)
Total Acquisition (Joules)	7428.4 (0.727)	9032.5 (0.821)	11701.1 (1.34)
Flight Allocation	0.22 (0.0001)	0.21 (0.0001)	0.19 (0.0001)
Reproductive Allocation	0.08 (0.0001)	0.14 (0.0001)	0.20 (0.0001)
Trade-off Acquisition (Joules)	2241.3 (0.423)	3145.0 (0.494)	4708.4 (0.936)
Trade-off Allocation	0.71 (0.0002)	0.61 (0.0002)	0.50 (0.0002)
Micropters (SW)			
DLM Mass (mg)	0.15 (0.003)	0.18 (0.003)	0.32 (0.004)
Ovary Mass (mg)	29.3 (0.062)	50.2 (0.106)	93.6 (0.204)
Remaining Mass (mg)	96.4 (0.100)	106.5 (0.108)	128.8 (0.136)
Total Acquisition (Joules)	6675.9 (5.28)	8124.0 (5.76)	11200.0 (11.14)
Flight Allocation	0.01 (0.0002)	0.01 (0.0002)	0.01 (0.0002)
Reproductive Allocation	0.20 (0.0004)	0.29 (0.0005)	0.38 (0.0007)
Trade-off Acquisition (Joules)	1457.2 (2.92)	2463.7 (5.01)	4585.9 (9.54)
Trade-off Allocation	0.05 (0.0008)	0.04 (0.0007)	0.05 (0.0007)

4.3.2 Genetics of phenotypic plasticity

Genetic correlations across environments were consistently high and near one (range: 0.60–1.09; Table 4.3). A genetic correlation that is significantly less than one indicates a significant genotype by environment interaction and significant genetic variation for plasticity. If the genetic correlation between environments is equal to one, the genetic basis for the trait is identical in the two environments and independent evolution in the two environments is constrained. After correction for multiple tests, our estimated correlations between environments only differed significantly from one in one case: the genetic correlation for trade-off acquisition between the 50% food treatment and the *ad libitum* treatment (χ^2_1 = 29.6, adjusted $P < 0.001$). Before adjusting for multiple tests, three additional cases were significant. The genetic correlation between the low food treatment and the *ad libitum* treatment differed from one for the traits ovary mass (χ^2_1 = 4.4, $P = 0.02$) and allocation to ovaries (χ^2_1 = 2.9, $P = 0.04$). And, the

Table 4.3: Genetic correlations and standard errors across pairs of environments.

Trait	**Low-50%**	**Low-*Ad***	**50%-*Ad***
DLM Mass	0.92 (0.09)	1.00 (0.08)	1.04 (0.05)
Ovary Mass	0.99 (0.09)	0.83 (0.09)†	0.94 (0.08)
Remaining Mass	1.02 (0.04)	1.02 (0.04)	1.03 (0.04)
Total Acquisition	1.09 (0.02)	0.98 (0.03)	0.95 (0.04)
Flight Allocation	0.87 (0.10)	0.96 (0.09)	1.01 (0.06)
Reproductive Allocation	0.94 (0.09)	0.85 (0.10)†	0.96 (0.08)
Trade-off Acquisition	0.96 (0.06)	0.60 (0.10)***	0.86 (0.08)†
Trade-off Allocation	0.86 (0.10)	0.88 (0.10)	0.94 (0.07)

Genetic correlation less than one: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ † $P < 0.05$ before adjustment for multiple tests.

genetic correlation between the 50% food and the *ad libitum* treatment differed from one for trade-off acquisition (χ^2_1 = 4.2, P = 0.02). These results suggest a low evolutionary potential for phenotypic plasticity.

4.3.3 Genetic parameters within environments

Phenotypic and additive variance components and heritabilities are summarized in Table 4.4. We found moderately high heritabilities for all the traits we measured (range: 0.19–0.52; Table 4.4) in all environments and the additive variance was always highly significant after correcting for multiple tests indicating a high evolutionary potential for all measured traits. The estimated heritabilities were consistently higher in the *ad libitum* food treatment in all but one case (remaining mass).

We calculated genetic correlations between organ masses separately from the genetic correlations between our measures of acquisition and allocation, because our estimates of acquisition and allocation are themselves derived from the organ masses (Table 4.5). Based on previous studies of the flight capability-reproduction trade-off (see Study System and references therein), we expect to find: 1) a negative genetic correlation between DLM mass and ovary mass, 2) a negative genetic correlation between ovary mass and remaining mass, and

Table 4.4: Additive genetic variances, residual variances and heritabilities for each trait in each food treatment. Adjusted *P* is the *P*-value for the significance of the additive genetic variance adjusted for multiple tests.

Traits	V_A	V_R	h^2	**Adjusted *P***
Low Food				
DLM Mass	5.3 (1.2)	19.7 (1.1)	0.21 (0.05)	<0.001
Ovary Mass	5.8 (1.3)	19.4 (1.1)	0.23 (0.05)	<0.001
Remaining Mass	9.6 (1.7)	15.8 (1.2)	0.38 (0.06)	<0.001
Total Acquisition	11.6 (1.9)	14.0 (1.3)	0.45 (0.06)	<0.001
Flight Allocation	4.8 (1.1)	20.2 (1.1)	0.19 (0.04)	<0.001
Reproductive Allocation	5.4 (1.3)	19.7 (1.2)	0.22 (0.05)	<0.001
Trade-off Acquisition	6.2 (1.3)	19.0 (1.2)	0.25 (0.05)	<0.001
Trade-off Allocation	4.8 (1.2)	20.3 (1.1)	0.19 (0.04)	<0.001
50% Food				
DLM Mass	6.5 (1.4)	18.8 (1.2)	0.26 (0.05)	<0.001
Ovary Mass	5.9 (1.4)	19.4 (1.2)	0.23 (0.05)	<0.001
Remaining Mass	9.5 (1.8)	16.0 (1.3)	0.37 (0.06)	<0.001
Total Acquisition	10.6 (1.8)	15.0 (1.3)	0.41 (0.06)	<0.001
Flight Allocation	6.1 (1.4)	19.1 (1.2)	0.24 (0.05)	<0.001
Reproductive Allocation	6.2 (1.4)	19.1 (1.2)	0.25 (0.05)	<0.001
Trade-off Acquisition	6.5 (1.4)	18.9 (1.2)	0.26 (0.05)	<0.001
Trade-off Allocation	6.6 (1.5)	18.8 (1.2)	0.26 (0.05)	<0.001
Ad libitum				
DLM Mass	7.1 (1.5)	18.2 (1.2)	0.28 (0.05)	<0.001
Ovary Mass	8.0 (1.5)	17.1 (1.2)	0.32 (0.05)	<0.001
Remaining Mass	8.7 (1.7)	16.7 (1.3)	0.34 (0.06)	<0.001
Total Acquisition	13.6 (2.2)	12.4 (1.4)	0.52 (0.07)	<0.001
Flight Allocation	7.1 (1.5)	18.1 (1.2)	0.28 (0.05)	<0.001
Reproductive Allocation	7.1 (1.4)	18.0 (1.2)	0.28 (0.05)	<0.001
Trade-off Acquisition	9.6 (1.7)	15.8 (1.3)	0.38 (0.06)	<0.001
Trade-off Allocation	7.9 (1.5)	17.2 (1.2)	0.31 (0.05)	<0.001

Table 4.5: Genetic correlations and standard errors between pairs of traits

Trait 1	**Trait 2**	**Low**	**50%**	***Ad libitum***
DLM	OV	-0.40 (0.13)*	-0.50 (0.05)**	-0.20 (0.14)
DLM	REM	0.70 (0.07)***	0.80 (0.05)***	0.80 (0.05)***
OV	REM	-0.002 (0.14)	-0.35 (0.13)*	0.12 (0.13)
AC	FA	-0.08 (0.14)	0.04 (0.14)	-0.14 (0.13)
AC	RA	0.19 (0.14)	-0.04 (0.14)	0.43 (0.11)**
FA	RA	-0.67 (0.09)***	-0.69 (0.08)***	-0.69 (0.08)***
TAC	TAL	0.09 (0.16)	-0.02 (0.16)	-0.51 (0.10)***

Genetic correlation greater than zero: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. DLM = dorso-longitudinal muscle mass, OV = ovary mass, REM = remaining mass, AC = total acquisition, FA = flight allocation, RA = reproductive allocation, TAC = trade-off acquisition, TAL = trade-off allocation.

3) a positive genetic correlation between DLM mass and remaining mass. A significant negative genetic correlation was present between ovary mass and DLM mass in the low and 50% food treatments, but this correlation was not significant in the *ad libitum* food treatment. The estimate of the genetic correlation between ovary mass and remaining mass was significantly negative in the 50% food treatment but was not significant in the low or *ad libitum* treatment. The genetic correlations between DLM mass and remaining mass were high and highly significant in all food treatments. The Y model, both in its original formulation (van Noordwijk and de Jong 1986) and Houle's (1991) explicitly genetic model, assumes acquisition and allocation are not correlated. The genetic correlation between total acquisition and reproductive allocation was significant only at the *ad libitum* food level, a pattern mimicked by the genetic correlation between trade-off acquisition and trade-off allocation. All other genetic correlations between our estimates of acquisition and allocation were not significant. Lastly, The genetic correlation between flight allocation and reproductive allocation was high, negative, and highly significant in all environments, reflecting the strong trade-off between these functions.

4.3.4 Changes in genetic architecture across environments

The genetic parameters varied significantly across the different food treatments (MANOVA: Wilks λ = 0.64, $F_{30,344}$ = 2.82, $P < 0.001$). We performed univariate ANOVAs for each genetic parameter with food treatment as the predictor variable. We found significant differences among food treatments for the covariance between total acquisition and reproductive allocation ($F_{2,186}$ = 4.0, P = 0.02) and for the covariance between trade-off acquisition and trade-off allocation ($F_{2,186}$ = 7.6, $P < 0.001$). These results indicate that the significant MANOVA is most likely due to differences in these parameters. Tukey's pairwise comparisons indicate the effect is due to changes in the genetic parameters between any food restriction (Low or 50%) and the *ad libitum* treatment. For the covariance between total acquisition and reproductive allocation, significant differences were found between the 50% and *ad libitum* treatment (adjusted P = 0.014). The covariance between trade-off acquisition and trade-off allocation was significantly different between the *ad libitum* treatment and both the low and 50% treatments (adjusted P = 0.001 and 0.007, respectively).

4.4 Discussion

4.4.1 Sources of variation in acquisition and allocation

A major goal of this study was to quantify the components of variation in acquisition and allocation. Phenotypic variance can be partitioned into genetic variance, environmental variance, and genotype by environment interaction (Lynch and Walsh 1998). We found significant additive genetic variance within environments for acquisition and allocation both measured at the level of the whole organism (total acquisition, flight allocation, and reproductive allocation) and at the level of the focal trade-off (trade-off acquisition and trade-off allocation). Significant levels of genetic variation in allocation are consistent with evidence from many other quantitative genetic studies of trade-offs (for reviews see Stearns 1989, 1992; Roff 1992, 2002b).

Large amounts of genetic variation in acquisition may be more surprising. The expectation is that selection should always favor individuals that acquire the most resources, because those individuals will be able to allocate more to all functions and will have the highest fitness. Strong and consistent selection for high acquisition ability should, in turn, lead to reduced additive genetic variance. Contrary to this expectation, we found the highest heritabilities for total acquisition. The presence of abundant genetic variation in acquisition leads to the question of what is maintaining variation in this trait. One possibility is that variation is maintained by a genotype by environment interaction (Gillespie and Turelli 1989). If a G×E does exist, the genotypes that acquire the most resources at low food levels differ from those that acquire the most resources at high food levels. This effect would indicate a trade-off between the ability to exploit high versus low resource environments. We did not find evidence for a G×E for total acquisition with all three pairwise genetic correlations being greater than 0.95 with small standard errors (0.04, Table 4.3). These results suggest that genotypes better at acquiring resources in one environment also tended to be better in the other environments. Mutation-selection balance has also been proposed as a mechanism that may maintain variation in acquisition (Houle 1991; Lynch and Walsh 1998). The lack of a significant G×E would be consistent with this mechanism as recurrent deleterious mutations would be expected to produce individuals with low fitness in all environments.

We did find a significant genotype by environment interaction for trade-off acquisition between the *ad libitum* food treatment and the 50% food treatment. This result means that the genotypes that devote the most to the flight capability and reproductive functions in the food restriction treatments are not always the same genotypes that devote the most to flight and reproduction in the *ad libitum* treatment. Because total acquisition does not show the same G×E, this effect is likely due to allocation to other functions that we did not measure. Extensions of the Y model including multiple traits have been developed (de Laguerie et al. 1991; de Jong 1993; Worley 2003). These models show that variation in the amount of resources individuals allocate to a third trait will cause variation in the size of the resource pool for two traits in a trade-off, producing the same effect as variation in total acquisition. At the level of the flight capability-reproduction trade-off, this genotype by environment interaction may serve to maintain genetic variation in this trait.

Two alternative hypotheses describe how the amount of genetic variation in acquisition will shift with changes in resource levels. Glazier (1999) hypothesized that variation in acquisition will be minimized in the less variable (but typically better) conditions of the laboratory. However, the opposite claim has also been made, that variation in acquisition may be minimized in low resource environments (Ernande et al. 2004; Messina and Fry 2003). We found that additive genetic variance in acquisition (both total acquisition and trade-off acquisition) increased with increasing food level, however this trend as tested by the MANOVA was not significant (Table 4.4; e.g., compare V_A in the three food food treatments for total acquisition and trade-off acquisition). This trend is consistent with the findings of Messina and Fry (2003), who found evidence for decreased variation in acquisition when seed beetles (*Callosobruchus maculatus*) were reared under food restriction. The authors found that the genetic correlation switched from being significantly negative under food restriction to being significantly positive in the presence of ample food (Messina and Fry 2003). According to the Y model, if genetic variance in acquisition is increased in higher food levels but genetic variance in allocation remains constant, we would expect to find a more positive genetic correlation (van Noordwijk and de Jong 1986; Houle 1991; de Jong and van Noordwijk 1992). In our study, the trend showed an increase in both the genetic variance in acquisition and the genetic variance in allocation. We found that the genetic correlation between DLM mass and ovary mass switched from being significantly negative in the food restriction environments to non-significant in the *ad libitum* environment. However, because genetic variance in both acquisition and allocation increased, it is not clear to what extent this result is due to an increase in acquisition.

Allocation also showed significant additive genetic variation. In addition, we found that allocation shifted with food level and that the response to food level was wing morph specific. As expected, SW individuals always allocated very little to flight, and, thus flight allocation and trade-off allocation did not change much with food level. Reproductive allocation did increase with food level, indicating that SW females allocated proportionally more of their total resources to reproduction as food level increased. When food is restricted, organisms must first meet maintenance demands to survive. When food levels rise above the levels required for maintenance, individuals may allocate these surplus resources to other functions. Thus, it is not surprising that proportional allocation to reproduction would increase with food level as individuals have an increasing surplus of resources. Macropterous (LW) individuals also allocated a greater proportion of resources to reproduction as food increased while the reverse was true for allocation to flight capability. As food level increased, proportional allocation to flight decreased, indicated by a decrease in both flight allocation and trade-off allocation. This effect is driven by the large increase in ovary mass (increase of 321%) compared to the smaller increases in DLM mass (34%) and remaining mass (40%) with food level. As food levels increase, LW females increase the amount they devote to reproduction much more dramatically than they increase the amount devoted to flight capability leading to a decline in the proportional allocation to flight capability.

These responses to food were very consistent across genotypes. A genetic correlation across environments that is less than one indicates a significant G×E. We found no evidence for genotype by environment interactions for organ masses or for allocation traits; nearly all genetic correlations across environments were greater than 0.9 and none were significantly different from one. This indicates that the evolution of plasticity in allocation is highly constrained and that the genetic basis of these traits is identical (or nearly identical) in the different food environments and that a response to selection in one food environment will influence the expression of that trait in other food environments. Essentially, in this system, all genotypes respond to increasing food in the same way, by increasing allocation to reproduction.

This result is perplexing in the face of a substantial amount of evidence of phenotypic plasticity in allocation in natural populations (see Introduction and references therein), given that a genetic correlation of one across environments is expected to prevent the evolution of phenotypic plasticity at least in the short term. In addition, this finding is unlikely to be a statistical artifact—not being able to detect a difference from a genetic correlation of one—because many of our estimates are equal to or very nearly equal to one (Table 4.3). In this system, very strong selection for the response to food may have lead to very little variance among genotypes in the response to food. Our results are consistent with an earlier quantitative genetic study of food restriction in which the genetic correlations across environments for DLM mass and ovary mass were found to be very high and not different from one (Roff and Gelinas 2003).

An additional possibility involves nymph versus adult environments. We only varied food levels for adults while all nymphs were provided with *ad libitum* food. If allocation decisions are fixed based on nymph food levels in this system, we would not see variation looking only across adult food levels. One would expect capital breeders, who utilize previously acquired, stored resources for reproduction (Bonnet et al. 1998) to base adult allocation decisions on developmental conditions. However, crickets are income breeders¬, meaning they use resources acquired during the reproductive period to fuel reproduction (Bonnet et al 1998). We generally expect income breeders to make adult allocation decisions based upon adult food conditions. Therefore, it would be interesting if we found evidence for nymphal food levels influencing adult allocation decisions. Future studies involving variation of nymphal food levels or possibly switching food levels between nymph and adult environments can elucidate this issue.

4.4.2 Genetic parameters within environments

While our results suggest independent evolution in two environments will be highly constrained, moderate to high evolutionary potential within environments is present for all traits indicating these traits can readily respond to selection. Within environments, trait evolution may be constrained by genetic correlations between traits. Previous studies have found a significant negative genetic correlation between DLM mass and ovary mass (Stirling et al. 1999; Roff 1994; Roff and Gelinas 2003). We also found a negative genetic correlation between these traits, though it was only significant in environments where food was restricted. This negative genetic correlation has been hypothesized to result from differential allocation of resources. In our study, we estimated proportional allocation to flight capability and reproduction. The genetic correlation between flight allocation and reproductive allocation was consistently strong, negative, and significant. This result is further evidence that these two functions compete for resources in this species.

Houle's (1991) extension of the Y model, which considers genetic variation in acquisition and allocation, assumes no pleiotropy between acquisition and allocation. If this assumption is correct, we expect to find no genetic correlation between these two traits. In the food restriction treatments (low and 50%), we found low, non-significant correlations between our measures of acquisition and allocation, indicating these traits can evolve independently. However, in the *ad libitum* food treatment, we found a significant positive genetic correlation between total acquisition and reproductive allocation and a significant negative genetic correlation between trade-off acquisition and trade-off allocation. These results are consistent with one another, because we measured trade-off allocation as allocation to flight capability. This correlation follows the general pattern we found across food treatments: individuals with higher values for acquisition allocate proportionally more to reproduction. In addition, when testing for differences in the genetic parameters between environments, we found a significant difference for the covariance between trade-off acquisition and trade-off allocation in the *ad libitum* compared with the food restriction treatments. This result indicates that at least in the *ad libitum* food treatment, some of the genes that determine resource acquisition also influence the proportional allocation to different functions. How pleiotropy between acquisition and allocation will influence the predictions of the Y model is an unexplored area.

4.4.3 Implications for trade-offs

This study highlights the importance of considering all potential sources of variation in acquisition and allocation when studying trade-offs. Shifts in trade-offs between environments may be due to changes in the genetic or phenotypic variance in these traits. In addition, the evolutionary trajectories of trade-offs will ultimately be governed by the evolution of the underlying processes of acquisition and allocation. However, few studies have focused directly on acquisition and allocation as traits. By doing so, we are able to advance our understanding of these traits in several important ways.

First, we showed substantial genetic variance in acquisition and allocation, and therefore these traits have the potential to evolve. These estimates can inform future models of the evolution of acquisition and allocation and how this evolution is expected to influence the evolution of trade-offs. Second, we showed that there was a significant genetic correlation between acquisition and allocation in the *ad libitum* treatment. Models of acquisition and allocation typically assume these traits are independent (van Noordwijk and de Jong 1986; Houle 1991). One reason these models make this assumption is because of a lack of empirical evidence showing pleiotropy. Our results provide this evidence and suggest that future models examine the effect of this non-independence on the Y model. Third, we showed that the genetic covariance between acquisition and allocation was significantly different in the different food environments. This result adds to the growing recognition that genetic parameters often shift when measured in different environments (Stearns et al. 1991; Hoffmann and Merila 1999; Srgo and Hoffmann 2004; Charmantier and Garant 2005). Fourth, we found that the trade-off, as measured by the genetic correlation between DLM mass and ovary mass, was only evident in the food restriction environments (Table 4.5). However, when measured directly as the genetic correlation between reproductive allocation and flight allocation, we found a consistent strong negative genetic correlation (Table 4.5). This result demonstrates that when allocation is measured independently of acquisition, we find evidence for the trade-off even when it may be obscured by variance in acquisition when measured only as the correlation between functions.

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Chapter 5

The interaction between acquisition and allocation of resources in *Gryllus firmus*: a test of the Y model

5.1 Introduction

The relationships between life history traits are hypothesized to be caused by two underlying processes: the acquisition of resources and the subsequent allocation of those resources (James 1974; Riska 1986; van Noordwijk and de Jong 1986; Reznick et al. 2000; Angiletta et al. 2003; Roff and Fairbairn 2007). Differential allocation to competing structures and functions generates "trade-offs" in the expression of these traits (Gadgil and Bossert 1970; Bell and Koufopanou 1985; Reznick 1985; Roff 1992, 2002; Stearns 1989). Allocation of more energy to one trait is hypothesized to reduce the energy available for the other traits, resulting in negative correlations between the competing traits. However, individuals also vary in the acquisition of resources (Ricklefs 1991; Weiner 1992; Hammond and Diamond 1997). Individuals that are able to acquire more resources than others will have more resources available to allocate to both traits. In the case where trait values depend more strongly on the total resource pool than the proportion allocated to competing traits, the correlation between traits will be positive. This concept has been formalized in a mathematical framework known as the "Y model" (James 1974; Riska 1986; van Noordwijk and de Jong 1986; Houle 1991).

The Y model was most clearly articulated by van Noordwijk and de Jong (1986). Their model consists of two traits (x_1 and x_2) drawing from a common resource pool and includes variation in both acquisition (the size of the total resource pool = T) and proportional allocation (proportion of resources allocated to $x_1 = P$) of resources. For a fixed acquisition value, variation in allocation leads to a negative covariance between traits. However, if some individuals in a population are able to acquire more resources than others, they will have a larger resource pool and can allocate more resources to both traits involved in a trade-off. Therefore, this variation in acquisition can lead to a positive correlation between these traits when measured across individuals in a population even while there is a functional trade-off within individuals. The Y model makes the important assumption that variances in acquisition and in allocation are independent. Specifically, this model predicts that the strength and sign of the covariance between the two traits depends on relative variation in acquisition and relative variation in allocation and can be predicted from the following equation (van Noordwijk and de Jong 1986):

$$\sigma_{x_1,x_2} = \sigma_T^2 \left(\mu_P \left(1 - \mu_P\right) - \sigma_P^2\right) - \mu_T^2 \sigma_P^2 \tag{5.1}$$

where σ_{x_1,x_2} is the covariance between x_1 and x_2, σ_T^2 is the variance in acquisition, σ_P^2 is the variance in allocation, μ_T is the mean of acquisition, and μ_P is the mean of allocation. From this equation it can been seen that if the variance of acquisition $\left(\sigma_T^2\right)$ is zero, the covariance will be negative $\left(-\mu_T^2\sigma_P^2\right)$. If the reverse is true and variance of allocation $\left(\sigma_P^2\right)$ is zero, the covariance will be positive $\left(\sigma_T^2\left(\mu_P\left(1-\mu_P\right)-\sigma_P^2\right)\right)$. We do not generally expect populations to show zero variance in either acquisition or allocation, therefore, two more informative predictions are:

1. For a constant μ_T, μ_P, and σ_P^2, increasing variance in acquisition $\left(\sigma_T^2\right)$ makes the covariance between x_1 and x_2 more positive.
2. For a constant μ_T, μ_P, and σ_T^2, increasing variance in allocation $\left(\sigma_P^2\right)$ makes the covariance between x_1 and x_2 more negative.

The Y model has been influential and is commonly cited as a possible explanation when expected trade-offs are not observed (e.g., Spitze et al. 1991; Genoud and Perrin 1994; Yampolsky and Ebert 1994; Reznick et al. 2000; Jordon and Snell 2002; Messina and Fry 2003; Ernande et al. 2004; Vorburger 2005). Despite its impact, the Y model has rarely been rigorously tested. This deficiency stems in part from the difficulties associated with accurately measuring the complex processes of acquisition and allocation in units of energy (see Chapter 3 and references therein). This task is difficult enough in a single population let alone for many different populations or species. Therefore, researchers aiming to test this model typically must make assumptions regarding acquisition and/or allocation. For example, Glazier (1999) attempted to test the Y model by comparing lab and field studies. He made the assumption that variation in acquisition will be minimized in the less variable conditions of the laboratory, and, therefore, negative correlations will be found more often in lab studies. This hypothesis was consistent with what he found in a review of the literature, and he concluded the Y model was supported. However, the opposite claim has also been made. Variation in acquisition may be minimized in low resource environments (Ernande et al. 2004; Messina and Fry 2003), and negative correlations may be found more often in harsher, more resource-stressed conditions, such as those in the field (Tuomi et al. 1983; Bell and Koufopanou 1985; Reznick 1985). Additionally, because the Y model predicts that the trade-off function will depend on the mean and variance of allocation as well, Glazier (1999) also assumes that the mean and variance of allocation are not also changing under laboratory conditions. Without explicitly measuring acquisition and allocation, we can only make hypotheses regarding the causes of changes in observed trade-off patterns.

Other researchers have attempted to test the Y model utilizing the trade-off between offspring size and offspring number and estimating acquisition and allocation from clutch mass (Christians 2000; Brown 2003). These authors simplified the above Y model predictions to a prediction regarding the variance ratio (reviewed in Roff and Fairbairn 2007). They predict that if the variance ratio (variance in allocation/variance in acquisition) increases, the correlation between offspring size and offspring number should decrease. However, it can be seen from equation 5.1 that this approach is an oversimplification. The covariance is not directly predicted from a comparison of the variances, and this prediction will only be valid when the means do not change. However, it is very unlikely that an increase in the mean will not be accompanied by an increase in the variance as larger measures will have a larger raw variance for an equal percent variability (Zar 2010). So, while both Christians (2000) and Brown (2003) were able to find support for the Y model with their simplified prediction, future tests must account for changes in the means and variances.

In this paper, we utilize the well-studied trade-off between flight capability and reproduction in the sand cricket, *Gryllus firmus* as a case study to test the Y model. We experimentally alter variation in acquisition by rearing individuals on three different food levels. We also estimate acquisition and allocation in units of energy in order to directly test the assumptions and predictions of the Y model. In addition, we propose principal components analysis as a new method to extract the underlying factors (acquisition and allocation) determining trade-offs.

5.2 Methods

5.2.1 Study system

Gryllus firmus is one of the most well studied wing dimorphic species and has emerged as a model system for the study of trade-offs. Like other wing dimorphic insects, *G. firmus* trade off reproductive output with flight capability (for reviews see Harrison 1980; Roff 1986; Dingle 1996; Zera and Denno 1997). Flight capable morphs are macropterous with long wings while flightless morphs are micropterous with short, non-functional wings. Even in the absence of flight, making and maintaining the large flight muscles incurs a significant energetic cost (Harrison 1980; Roff 1984; Roff 1986; Dingle 1996; Zera and Denno 1997; Zera et al. 1997; Crnokrak and Roff 2002). Macropterous individuals have significantly larger dorso-longitudinal muscles (hereafter DLMs), the main flight muscles, than micropterous individuals while micropterous individuals begin reproducing earlier and have a higher cumulative fecundity (Roff 1984, 1994; Zera et al. 1997; Roff and Gelinas 2003). This trade-off also exists within macropterous morphs as individuals with larger DLMs tend to have smaller ovaries (Roff et al. 2002; Roff and Gelinas 2003). Most macropterous individuals histolyze their flight muscles within the first two weeks following eclosion into the adult form (Fairbairn and Roff 1990; Stirling et al. 2001) and fecundity following this histolysis increases dramatically because the resources otherwise devoted to flight muscle are reallocated to egg production (Roff 1989). Many physiological studies have also demonstrated differences in the allocation of available nutrients in flight capable compared to flightless morphs (Zera et al. 1994; Zera and Denno 1997; Zera and Brink 2000; Zera 2005; Zhao and Zera 2006), providing a strong argument that these two traits are in a functional trade-off and compete for resources.

5.2.2 Rearing Protocol

The rearing protocol for this experiment is described in detail in Chapter 4. Briefly, individuals from 63 half-sibling families were reared under three different diet treatments. Parents for these families were chosen at random from a stock population started from 27 females (assumed to be multiply mated) collected in September 2007 from Gainesville, FL (Lat.: N 29.68°, Lon.: W 82.27°). Once hatched, nymphs from each family were split between two 1.8 Liter buckets to control for microenvironmental effects. Nymphs were provided with ground LabDiet Prolab© Rabbit Chow *ad libitum*. On the day of their final ecdysis (final molt to the adult form), individuals were placed in individual 0.5 Liter cages and assigned to one of three food treatments. The high food treatment consisted of ad libitum LabDiet Prolab© Rabbit Diet, the 50% diet consisted of a daily ration of food determined to reduce ovary mass by 50% (E. King *unpublished data*), and the low food treatment consisted of a daily ration of food just above the amount required for survival for 20 days (Crnokrak and Roff 1998). All cages were kept at 28° C and 50% relative humidity in environmentally controlled growth chambers (ECG; Chagrin Falls, Ohio). Up to ten females from each family in each food treatment were preserved in 70% ethanol at seven days of age (past the final ecdysis) for future dissection. While mortality occasionally prevented the collection of the full ten individuals (mean = 9.1, sd = 1.5), the data were fairly well balanced and thus, for the present analysis, we ignored the family structure.

Each cricket was dissected and the dorso-longitudinal muscles and ovaries were removed, dried and weighed to the nearest 0.0001 g. The state of muscle histolysis was also scored for each dissected cricket using a three-level scale (no evidence of histolysis, partially histolyzed, and totally histolyzed or absent). In this study, individuals with totally histolyzed or absent muscles were excluded, because we are most interested in the direct trade-off between ovary mass and DLM mass. Because individuals with totally histolyzed muscles completely lack DLMs, they cannot be used to examine the relationship between variation in DLM mass and ovary mass. A previous study demonstrated that the energy allocated to reproduction and flight capability can be estimated from the masses of ovaries and flight muscles (see Chapter 3 and below). Therefore, we also estimated total acquisition and allocation to flight capability and reproduction using the following methods.

We estimated the amount of energy allocated to reproduction by, incorporating the energy content, synthesis costs, and maintenance costs of ovaries according to the model developed in Chapter 3. Similarly, the amount of energy allocated to flight capability was estimated from DLM mass according to the model developed in Chapter 3 incorporating energy content, synthesis costs, maintenance costs, and the energy stored for flight. In the Y model, only a single trade-off relationship is considered. Therefore, the appropriate total resource pool is actually the total resource pool available only for that trade-off. More complex systems such as hierarchical resource allocation or those involving more than two traits require a more complex model (de Laguerie et al. 1991; de Jong 1993; Worley 2003). For this study, we are interested only in the trade-off between flight capability and reproduction, and we therefore define the resource pool (i.e., acquisition) as the total energy allocated to reproduction + the total energy allocated to flight capability.

5.2.3 Extracting the underlying determinants of resource based trade-offs: A principal components approach

In the case where acquisition and allocation do vary independently and are the true determinants of the covariance between two traits in a resource-based trade-off (as predicted by the Y model), a principal components analysis should successfully extract these underlying components. Specifically, we would expect principal components analysis to extract a component corresponding to variance in acquisition and a component corresponding to variance in allocation. We tested this prediction by performing a principal components analysis of DLM mass and ovary mass followed by a regression of our estimated values for acquisition and allocation on the scores of the principal components for both the full data set and for individual food levels. Whether the first component corresponds to acquisition or allocation will depend upon the dominant source of variation. If variation in acquisition dominates, the first component will correspond to acquisition and the second will correspond to allocation. But, if variation in allocation dominates, the first component will correspond to allocation and the second will correspond to acquisition. The loadings of the variables on the components will inform how the variables relate to the components. If both variables load together (both positive or both negative), the component is hypothesized to correspond to acquisition. If the variables load with opposite signs (one positive and the other negative), the component is hypothesized to be allocation.

5.3 Results

5.3.1 Predictions 1 and 2

1. For a constant μ_T, μ_P, and σ_P^2, increasing variance in acquisition $\left(\sigma_T^2\right)$ makes the covariance between x_1 and x_2 more positive.
2. For a constant μ_T, μ_P, and σ_T^2, increasing variance in allocation $\left(\sigma_P^2\right)$ makes the covariance between x_1 and x_2 more negative.

In our study, we experimentally alter variation in acquisition by rearing individuals on three different food levels. We can test prediction 1 by comparing the correlation measured across food levels with the correlations within food levels. Specifically, we predict that correlations across food levels will be more positive because variance in acquisition will be high. In contrast, within each food level we predict more negative correlations because variance in acquisition will be lower. Prediction 2 can be tested by restricting variation in allocation. This restriction is easily achieved in this system due to our measurement of the state of muscle histolysis. By including only individuals with fully developed, non-histolyzed muscles in the analysis, we effectively constrain variation in allocation. We can then compare the correlations from the data set including only individuals with fully developed DLMs with the correlations from the full data set, predicting a higher (more positive) correlation in the former.

To confirm these changes in the relative variation in acquisition and allocation, we can use the coefficient of variation. The coefficient of variation is the standard deviation scaled to the mean and is typically a better estimate of the true variability in a sample than the raw variance (Zar 2010). This is because the highest food level will have a higher mean value for acquisition and will therefore also have a larger raw variance. The coefficient of variation is a relative measure of variance, scaled to the mean of the data and therefore, it will typically be less influenced by changes in the mean than the raw sample variance. One case where the coefficient of variation is not expected to perform well is when the mean is near zero. In this case, small changes in the mean have large effects on the coefficient of variation. Because we measure allocation as a proportion, this is a potential problem. We used a simulation to show that the coefficient of variation for a proportion will be most influenced by changes in the mean when the mean is below 0.2 (data not shown). Our mean values are all above 0.5 and therefore will not be strongly influenced by changes in the mean. We used a Bonferroni procedure to estimate simultaneous confidence intervals described by Miller and Feltz (1997) as a multiple comparisons test for differences between our estimated coefficients of variation. We also tested for differences in the raw sample variance using the multiple comparisons procedure outlined in Zar (2010). We can then compare the observed correlations to changes in the coefficients of variation in acquisition and allocation.

We computed Pearson's correlation coefficients between DLM mass and ovary mass for the following data sets (Figure 5.1): 1) the full data set including all individuals across all food levels, 2) all individuals in the low food treatment, 3) all individuals in the 50% food treat-

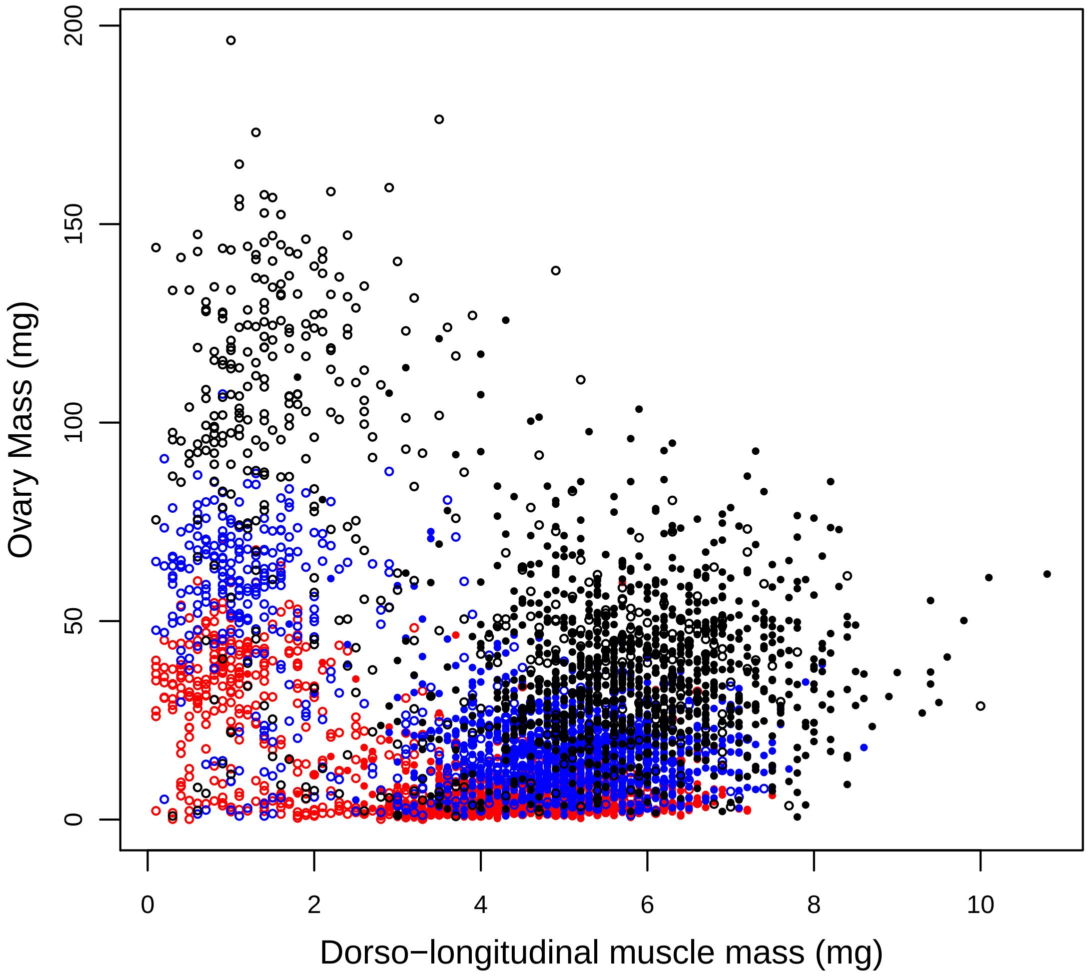

Figure 5.1: Ovary mass (mg) versus dorso-longitudinal muscle mass (mg) by food level and DLM histolysis status. Low food = red, 50% food = blue, *Ad libitum* = black. Open circles = partially histolyzed DLMs, Closed circles = non-histolyzed DLMs.

Table 5.1: Comparison of the variation in acquisition (T) and allocation (P) and the correlation between dorso-longitudinal muscle mass (x_1) and ovary mass (x_2) for different data sets.

Data Set	**N**	σ_T^2	σ_P^2	**CV**$_T$	**CV**$_P$	**r**$_{x_1,x_2}$
Full Data Set						
All Food Levels	4148	1.88 (a)	0.068 (a)	0.40 (a)	0.39 (a)	-0.31 (a)
Low Food	1392	0.37 (b)	0.067 (a)	0.26 (b)	0.33 (b)	-0.63 (b)
50% Food	1377	0.51 (b)	0.060 (a)	0.22 (c)	0.37 (a)	-0.67 (b)
Ad libitum	1379	1.73 (a)	0.049 (b)	0.28 (b)	0.41 (a)	-0.57 (b)
Non-histolyzed DLMs only						
All Food Levels	2481	1.46 (c)	0.024 (c)	0.34 (d)	0.21 (c)	0.31 (c)
Low Food	751	0.25 (d)	0.007 (d)	0.21 (c)	0.10 (d)	-0.02 (d)
50% Food	831	0.36 (b)	0.012 (e)	0.19 (c)	0.15 (e)	-0.13 (d)
Ad libitum	899	1.13 (e)	0.016 (f)	0.23 (c)	0.20 (c)	-0.03 (d)

Note: N = sample size, σ_T^2 = variance in acquisition, σ_P^2 = variance in allocation, CV$_T$ = coefficient of variation in acquisition, CV$_P$ = coefficient of variation in allocation. Different letters in parentheses indicate significant differences.

ment, 4) all individuals in the ad libitum treatment, 5) individuals with non-histolyzed muscles across all food levels, 6) individuals with non-histolyzed muscles in the low food treatment, 7) individuals with non-histolyzed muscles in the 50% food treatment, and 8) individuals with non-histolyzed muscles in the ad libitum food treatment. We can test for differences between these estimated correlation coefficients by using Fisher's *z* transformation followed by Tukey's multiple comparisons (Zar 2010). These results are summarized in Table 5.1.

Prediction 1

The correlations between ovary mass and dlm mass within food treatments did not differ significantly from one another for the full data set or for the data set restricted to non-histolyzed DLMs (Table 5.1) and most are significantly negative (Table 5.2). As predicted, the coefficient of variation in acquisition is significantly higher across food treatments than within food treatments. For both data sets, the correlation across food treatments was also

Table 5.2: Correlations between x_1 and x_2 and their associated *P*-values.

Data Set	**N**	$\mathbf{r}_{x_1,x_2}$	***P***
Full Data Set			
All Food Levels	4148	-0.31	< 0.001
Low Food	1392	-0.63	< 0.001
50% Food	1377	-0.67	< 0.001
Ad libitum	1379	-0.57	< 0.001
Non-histolyzed DLMs only			
All Food Levels	2481	0.31	< 0.001
Low Food	751	-0.02	0.56
50% Food	831	-0.13	< 0.001
Ad libitum	899	-0.03	0.39

significantly more positive than the correlations within food treatments as would be expected by the Y model (Table 5.1).

Prediction 2

The coefficients of variation in allocation for the data set including only individuals with non-histolyzed DLMs are all significantly lower than those for the full data set, and the raw variances follow the same pattern (Table 5.1). As predicted, this decrease in variability in allocation corresponded to significantly less negative correlations within food treatments for the data set restricted to individuals with non-histolyzed DLMs compared to those for the full data set (Table 5.1). In addition, the correlation across food treatments from the restricted data set is significantly more positive than the correlation across food treatments for the full data set (Table 5.1). The correlation for the data set in which variation in allocation is restricted (only non-histolyzed DLMs) but variation in acquisition is inflated (across food levels) is of particular interest because it is significantly positive (Table 5.2) and completely obscures the trade-off, as predicted by the Y model.

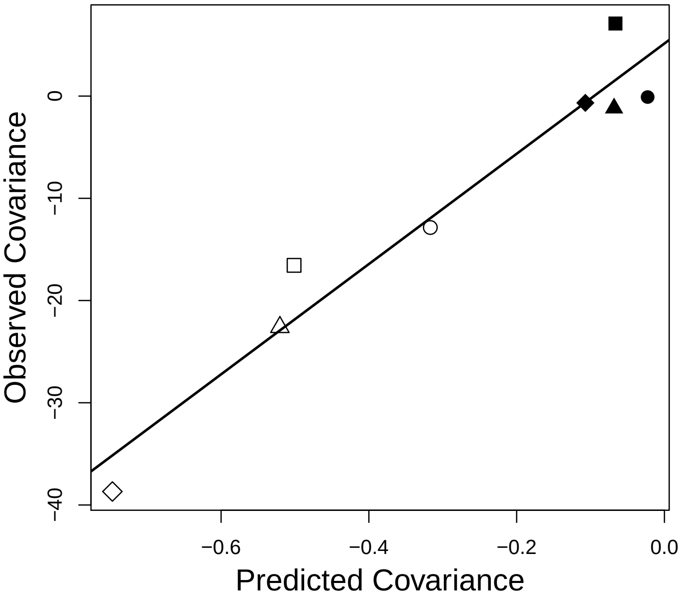

Figure 5.2: The observed covariance between dorso-longitudinal muscle mass and ovary mass versus the predicted covariance from equation 5.1. Open symbols = Full data set, Closed symbols = Non-histolyzed DLMs only, Squares = All food levels, Circles = Low food, Triangles = 50% food, Diamonds = *Ad libitum* food.

5.3.2 Does equation 5.1 accurately predict the covariance between dorso-longitudinal muscle mass and ovary mass?

The tests above took advantage of marked changes in the percent variability in acquisition and allocation. However, we know that more subtle changes in both the mean and the variance of acquisition and allocation will influence the observed covariance. We can test the performance of the predicted relationship between acquisition and allocation and the covariance by comparing predicted values from equation 5.1 with our observed values for all the examined datasets. Equation 5.1 performs very well: the predicted values explain 94% of the variance in the observed covariance as measured by linear regression (R^2_6 = 0.94, $P < 0.001$; Figure 5.2).

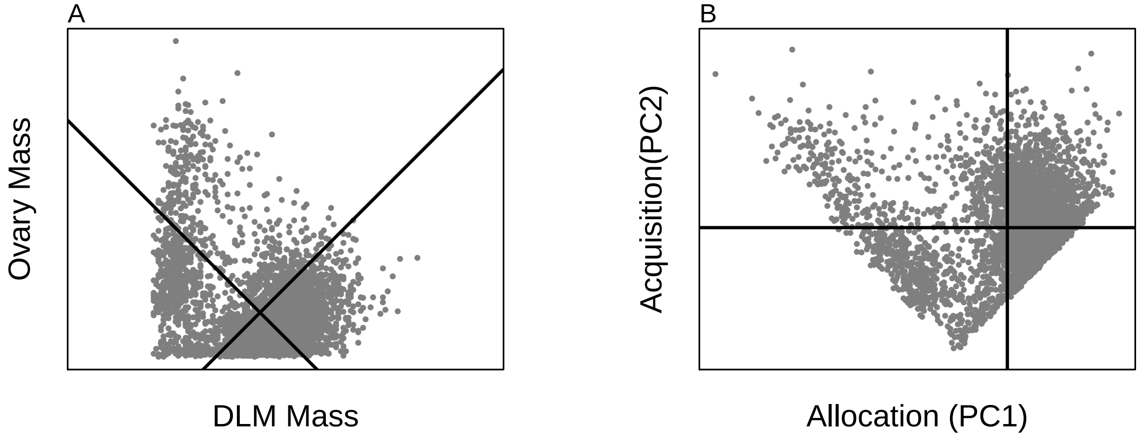

Figure 5.3: Graphical representation of a principal components analysis on DLM mass and ovary mass for the full data set. A) DLM mass versus ovary mass with lines representing the two principal components. B) Principal component one (hypothesized to be allocation) versus principal component two (hypothesized to be acquisition).

5.3.3 Are acquisition and allocation independent as assumed by the Y model?

A major assumption of the Y model is that the acquisition of resources and the allocation of resources are independent. We tested this assumption by testing if acquisition and allocation were significantly correlated within and across food levels. The null expectation of a correlation of zero was first confirmed with a simulation model (data not shown). The proportion of resources allocated to flight capability was significantly correlated with total acquisition in all examined data sets (All food levels: r_{4146} = -0.46, $P < 0.001$, Low food: r_{1390} = 0.07, P = 0.009, 50% food: r_{1375} = -0.26, $P < 0.001$, *ad libitum* food: r_{1377} = -0.56, $P < 0.001$). The relationship between acquisition and allocation was weaker within the lower food levels and while still significant, was near zero in the low food treatment.

5.3.4 Principal components analysis

For our data sets, we predicted the first principal component would capture the variance due to variance in allocation, because all correlations from the full data set were negative and therefore the first component will represent the negative relationship between DLM mass and ovary mass. The second principal component is independent of the first and should correspond to variance in acquisition. If acquisition and allocation are independent, there will either be no or only a weak relationship between 1) acquisition and the first principal component (the component hypothesized to be allocation) and 2) allocation and the second principal component (the component hypothesized to be acquisition).

Principal components analysis performed very well (Figure 5.3, Table 5.3). The variance in acquisition was captured by the second principal component and this explained at least 82% of the variance in our observed acquisition. There was only a weak relationship between PC2 and allocation ($R^2 < 0.07$). The variance in allocation was captured in PC1 which explained at least 76% of the variance in observed allocation. It explained less than 19% of the variance in observed acquisition. The percentage of the variance in the data set explained by each component is given in Table 5.3. The hypothesized allocation component explained 78.4–83.5% of the variance in DLM mass and ovary mass while acquisition explained 16.5–21.6% of the variance within food treatments. Across food treatments, allocation explained 65.5% of the variance while acquisition explained 34.5%. These results are also consistent with the expectations of the Y model with allocation being less dominant across food treatments than within food treatments.

Table 5.3: R^2 values from the regression of observed values for acquisition and allocation and the scores from a principal components analysis of dorso-longitudinal muscle mass and ovary mass. $P < 0.001$ for all values.

	Component 1		**Component 2**	
Data Set	*Acquisition*	*Allocation*	*Acquisition*	*Allocation*
All Food Levels	0.08	0.76	0.92	0.06
Low Food	0.06	0.89	0.94	0.03
50% Food	0.01	0.91	0.99	0.03
Ad libitum	0.18	0.88	0.82	0.03

5.4 Discussion

We found strong support for the predictions of the Y model in this system. The correlation between DLM mass and ovary mass was less negative in the data set where we experimentally inflated variability in acquisition by rearing individuals on different food levels as predicted by the Y model. In addition, for the data set where we restricted variability in allocation by considering only individuals with non-histolyzed muscles, the correlation also became less negative as predicted. Despite these changes, most correlations remained negative, indicating that variability in allocation was still the dominant influence. The only data set where we observed a positive correlation was for the data set across food levels (increased variability in acquisition) with only individuals with non-histolyzed DLMS (reduced variability in allocation). Previous tests have also found support for the predictions of the Y model, but they have had to make various assumptions regarding acquisition and/or allocation (see Introduction and references therein). We estimated acquisition and allocation in units of energy and compared these estimates to the predictions of the Y model as a direct test of the Y model.

We also tested an assumption of the Y model: that acquisition and allocation are independent of one another. We found that acquisition and allocation were significantly correlated in all of our examined data sets, though the highest correlations were found across food levels and at the high food treatment. The correlation across food levels indicates that *G. firmus* shows phenotypic plasticity in allocation in response to changes in resource availability. Individuals reared on higher food levels tend to allocate proportionally less to flight capability. This relationship was maintained within ad libitum and 50% food levels where individuals that were able to acquire more resources tended to allocate proportionally less to flight capability. The Y model still performed well when predicting the covariance between DLM mass and ovary mass despite the violation of the assumption of independence. Christians (2000) also found that acquisition and allocation were correlated in his test of the Y model in waterfowl, however he was also still able to find support for the predictions of the Y model. Thus, the Y model seems to be robust to the assumption of independence at least for moderate correlations between acquisition and allocation. However, the degree to which the predictions of the Y model are expected to change if the assumption of independence is relaxed has yet to be explored and could be the subject of future theoretical work.

Cases exist where we would not expect acquisition and allocation to be independent. One case is when variation in acquisition influences the evolution of allocation strategies. There are many examples of allocation strategies shifting in response to changes in acquisition regime (e.g., Gebhardt and Stearns 1988; Kaitala 1991; Chippendale et al. 1993; Ellers and van Alphen 1997; Blanckenhorn 1998; Tessier et al. 2000; Jordan and Snell 2002; Czesak and Fox 2003; Messina and Fry 2003; Ernande et al. 2004; Bashey 2006; Ruf et al. 2006), and recent models have begun to explore when environmental variation in resource availability will lead to the evolution of phenotypic plasticity in allocation (e.g., Fischer et al. 2009; King and Roff 2010). In addition, individuals must allocate resources to acquisitive ability and thus acquisitive ability itself may trade off with other functions. Indeed some researchers have found evidence for these types of trade-offs (e.g., Ricklefs 1991; Weiner 1992; Leroi et al. 1994; Hammond and Diamond 1997; Tessier and Woodruff 2002). Of course, in this case, allocation to acquisitive ability will directly influence the size of one's resource pool and acquisition and allocation certainly could not be assumed to be independent. A promising line of future research is how and when these two processes will influence each other's evolution and how this will influence trade-off relationships.

5.4.1 Principal components analysis as a method to extract acquisition and allocation

Acquisition and allocation are very challenging to quantify (see Chapter 3 and references therein) and this is a significant barrier to researchers interested in the underlying processes determining trade-offs. Principal components analysis (PCA) performed very well in this system when used to extract the traits acquisition and allocation. These two processes, acquisition and allocation of resources are hypothesized to be the underlying factors determining the relationship between traits in a functional trade-off. Principal components analysis is designed to extract the patterns of correlations among variables and these may correspond to underlying processes creating the correlations between the variables (Tabachnick and Fidell 2001). PCA is therefore ideal as a method to estimate acquisition and allocation. This method does make the assumption that acquisition and allocation are independent of one another. We found that for this system, PCA performed well even when acquisition and allocation were moderately correlated, however when these two processes are highly correlated, PCA is not expected to perform well as the extracted components are orthogonal to one another. Future theoretical work could address how well PCA extracts acquisition and allocation under various conditions.

This method has two major potential advantages. First, the variances of the acquisition component and the allocation component can be directly compared because the variances are standardized. Therefore, from the variances in the components, one can hypothesize about the relative contributions of acquisition and allocation to the trade-off relationship. For example, in the PCA reported here, allocation explained between 66% and 84% of the variance in

Table 5.4: Percent of variance explained by each principal component.

	% Variance Explained	
	Component 1 *"Allocation"*	*Component 2* *"Acquisition"*
All Food Levels	65.5	34.5
Low Food	81.5	18.5
50% Food	83.5	16.5
Ad libitum	78.4	21.6

DLM mass and ovary mass, while acquisition explained between 17% and 35% of the variance (Table 5.4). This comparison is very different from other estimates of acquisition and allocation where allocation is measured as a proportion and acquisition can be measured in many different units depending on how it is estimated. Also, this comparison casts the Y model in the way it is best understood—as a model comparing the contribution of variance in acquisition to the contribution of variance in allocation (Roff and Fairbairn 2007). Second, the scores of the principal components can be treated as trait values for acquisition and allocation and used to test hypotheses about acquisition and allocation themselves. This may be particularly useful when asking questions about allocation in systems where it is difficult to conduct analyses using proportions. Of course, this method is dependent on the same assumptions of the Y model itself—that acquisition and allocation are independent and that the focal trade-off is resource-based. However, when a trade-off is already assumed to be governed by the Y model, PCA may be the best method to estimate the underlying processes of acquisition and allocation.

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Conclusions

The covariation between acquisition and allocation of resources has been identified as an important determinant of variation in trade-offs (James 1974; Riska 1986; van Noordwijk and de Jong 1986; Houle 1991). In this dissertation, I used a combined approach of mathematical modeling, physiological assays and quantitative genetics to address several key unanswered questions concerning genetic and phenotypic variation in acquisition and allocation. My findings have implications for our understanding of adaptive significance of phenotypic plasticity in allocation, the physiology and evolutionary dynamics of trade-offs, the importance of all sources of variation in acquisition and allocation, and the significance of that variation in determining trade-off patterns.

In chapter one, I demonstrated the evolvability of an important allocation decision—wing morphology induction—in the water strider, *Aquarius remigis*. This finding lends support to previous studies on the evolution of wing dimorphism in water striders that have assumed wing dimorphism has the potential to evolve (e.g., Vepsäläinen, 1978; Andersen, 1982, 2000; Fairbairn, 1988; Kaitala and Dingle, 1992; Ahlroth et al. 1999; Pfenning and Poethke, 2006). I also showed that the reaction norm of wing morphology in response to temperature has the potential to evolve. This study is the first demonstration that both proportion winged and its response to environmental cues have the potential to adapt to environmental conditions. It will shape future studies of the adaptive significance and evolution of wing dimorphisms by highlighting the role of adaptive plasticity in evolutionary responses.

In chapter two, I developed novel predictions for how and when phenotypic plasticity in resource allocation will evolve in response to variation in resource availability in wing dimorphic insects. Dispersal is particularly important in heterogeneous environments but is often energetically expensive, taxing organisms' energy budgets (Dingle 1996). When resource levels vary in the environment, organisms must decide how much to allocate to dispersal in favorable versus unfavorable resource environments. The models developed in this chapter predict that the temporal autocorrelation between patches (i.e., how predictable the resource level in a patch is over time) is a key factor in the pattern of plasticity in resource allocation that evolves. Specifically, selection favors higher investment in flight under poor conditions in predictable environments and lower investment in unpredictable environments. These models are some of the first to show how phenotypic plasticity in resource allocation is expected to evolve.

In chapter three, I demonstrated that the energy allocated to reproduction and flight capability can be estimated from the masses of ovaries and flight muscles in a population of *Gryllus firmus*. Estimating total energy acquisition and energy allocation to various functions presents a significant challenge to researchers. However, trade-off patterns are expected to be related to the relative variances in energy acquisition and energy allocation (van Noordwijk and de Jong 1986; Reznick et al. 2000), and, therefore, estimating these quantities is critical for studies of the energetic basis of trade-offs. In *G. firmus*, it has been well established that wing morphs differentially allocate their resources toward flight capability and reproduction, however my study is the first to explicitly elucidate the relationship between variation in energy allocation and variation in tissue masses. The ability to estimate relative acquisition and allocation from simple mass measurements significantly simplified the experiments described in chapters 4 and 5 where detailed biochemical measurements were not feasible.

In chapter four, I described a large scale breeding experiment with *Gryllus firmus* families split over several resource levels. I quantified the major components of variation in acquisition and allocation: genetic variance, environmental variance, and genotype by environment interaction. This study highlighted the importance of considering all potential sources of variation in acquisition and allocation when studying trade-offs. I found a significant genetic variance for both acquisition and allocation, but I also found that independent evolution in different resource environments will be constrained. These are the first direct estimates of the genetic architecture of acquisition and allocation.

In the final chapter, I utilized *Gryllus firmus* to test the assumptions and predictions of the Y model described by van Noordwijk and de Jong (1986). This study is the first to use estimates acquisition and allocation to directly test the Y model. An assumption of the Y model, that acquisition and allocation will be independent of one another, was not supported. However, despite this result, I found strong support for the predictions of the Y model in this system, demonstrating the Y model is robust to violations of the assumption of independence. In this chapter, I used principal components analysis to estimate acquisition and allocation, and found that this novel methodology performed very well with this data set. This method will be invaluable to researchers aiming to estimate acquisition and allocation in trade-offs hypothesized to be resource-based.

These findings also suggest several avenues of future research. First, in chapters four and five I found that acquisition and allocation were not independent, as assumed by the Y model. Future theoretical work should examine the degree to which the predictions of the Y model are expected to change if the assumption of independence is relaxed. Second, the evolutionary trajectories of trade-offs will ultimately be governed by the coevolution of acquisition and allocation. In chapter two, I showed that variation in allocation may evolve to depend upon variation in acquisition. This finding introduces a level of complexity to our understanding of the evolution of trade-offs, and is an important avenue for future research. Future models of the evolution of phenotypic plasticity in allocation may also incorporate allocation of resources to acquisitive ability itself, which would feed back to the size of an individual's resource pool. An understanding of how the evolution of resource acquisition and subsequent resource allocation may be linked is key to understanding the evolution of trade-offs in general. Third, I found that principal components analysis (PCA) could be used to estimate acquisition and allocation in this system. Additional work is needed to determine the general conditions where PCA will successfully approximate the underlying processes determining resource-based trade-offs. By understanding the mechanisms determining variation in trade-off patterns, we will gain a better understanding of their true role in the evolution of traits.

5.6 References

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Appendix A

Estimates of genetic parameters for *Gryllus firmus* females in three food treatments with wing morphology included as a fixed effect

Table A.1: Genetic correlations and standard errors across pairs of environments

Trait	**Low–50%**	**Low–*Ad***	**50%–*Ad***
DLM Mass	0.94 (0.08)	1.01 (0.07)	1.04 (0.06)
Ovary Mass	0.99 (0.08)	0.82 (0.09)*	0.98 (0.08)
Remaining Mass	1.05 (0.04)	1.00 (0.04)	1.00 (0.04)
Total Acquisition	1.08 (0.03)	0.96 (0.03)	0.93 (0.04)
Flight Allocation	0.90 (0.10)	0.97 (0.09)	1.02 (0.07)
Reproduction Allocation	0.97 (0.09)	0.84 (0.09)*	1.00 (0.08)
Trade-off Acquisition	1.00 (0.05)	0.66 (0.09)**	0.87 (0.07)*
Trade-off Allocation	0.91 (0.11)	0.88 (0.10)	0.96 (0.07)

Genetic correlation less than one: *$P < 0.05$, **$P < 0.01$ before adjustment for multiple tests.

Table A.2: Additive genetic variances, phenotypic variances and heritabilities for each trait in each food treatment. Adjusted *P* is the *P*-value for the significance of the additive genetic variance adjusted for multiple tests.

Traits	V_A	V_P	h^2	**Adjusted *P***
Low Food				
DLM Mass	2.4 (0.50)	11.1 (0.40)	0.21 (0.04)	<0.001
Ovary Mass	4.3 (0.89)	18.6 (0.68)	0.24 (0.04)	<0.001
Remaining Mass	204.1 (33.4)	539.8 (21.6)	0.38 (0.05)	<0.001
Total Acquisition	2.8 (0.42)	5.9 (0.25)	0.48 (0.06)	<0.001
Flight Allocation	19.1 (4.3)	107.0 (3.8)	0.18 (0.04)	<0.001
Reproduction Allocation	19.3 (4.1)	88.6 (3.2)	0.22 (0.04)	<0.001
Trade-off Acquisition	5.8 (1.05)	19.4 (0.74)	0.29 (0.05)	<0.001
Trade-off Allocation	4.3 (1.0)	24.9 (0.89)	0.17 (0.04)	<0.001
50% Food				
DLM Mass	3.7 (0.79)	16.3 (0.60)	0.24 (0.04)	<0.001
Ovary Mass	2.5 (0.60)	13.5 (0.49)	0.20 (0.04)	<0.001
Remaining Mass	245.6 (42.0)	731.9 (28.7)	0.34 (0.05)	<0.001
Total Acquisition	3.0 (0.45)	7.1 (0.27)	0.43 (0.06)	<0.001
Flight Allocation	15.0 (3.4)	74.9 (2.7)	0.21 (0.04)	<0.001
Reproduction Allocation	12.3 (2.8)	62.8 (2.3)	0.19 (0.04)	<0.001
Trade-off Acquisition	9.3 (1.6)	28.3 (1.1)	0.31 (0.05)	<0.001
Trade-off Allocation	3.2 (0.74)	16.5 (0.60)	0.20 (0.04)	<0.001
Ad libitum				
DLM Mass	7.6 (1.5)	30.4 (1.1)	0.27 (0.05)	<0.001
Ovary Mass	3.3 (0.62)	12.1 (0.46)	0.27 (0.05)	<0.001
Remaining Mass	487.8 (82.5)	1353.7 (54.5)	0.36 (0.05)	<0.001
Total Acquisition	9.6 (0.93)	19.1 (0.85)	0.50 (0.06)	<0.001
Flight Allocation	11.3 (2.3)	48.7 (1.9)	0.24 (0.04)	<0.001
Reproduction Allocation	12.1 (2.4)	49.1 (1.8)	0.25 (0.04)	<0.001
Trade-off Acquisition	35.6 (6.1)	98.1 (4.0)	0.36 (0.05)	<0.001
Trade-off Allocation	2.9 (0.52)	10.0 (0.38)	0.29 (0.05)	<0.001

Table A.3: Genetic correlations and standard errors between pairs of traits

Trait 1	**Trait 2**	**Low**	**50%**	***Ad libitum***
DLM	OV	-0.33 (0.13)*	-0.36 (0.14)*	-0.16 (0.14)
DLM	REM	0.68 (0.08)***	0.73 (0.07)***	0.80 (0.05)***
OV	REM	0.05 (0.14)	-0.21 (0.14)	0.12 (0.13)
AC	FA	-0.09 (0.14)	0.00 (0.14)	-0.05 (0.13)
AC	RA	0.25 (0.13)	0.10 (0.14)	0.39 (0.11)**
FA	RA	-0.62 (0.10)***	-0.58 (0.10)***	-0.67 (0.08)***
TAC	TAL	-0.03 (0.16)	-0.02 (0.15)	-0.40 (0.11)**

Genetic correlation greater than zero: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. DLM = dorso-longitudinal muscle mass, OV = ovary mass, REM = remaining mass, AC = total acquisition, FA = flight allocation, RA = reproductive allocation, TAC = trade-off acquisition, TAL = trade-off allocation.