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MATERNAL EFFECTS AND SEXUAL SELECTION IN THE HELICONIA BUG, LEPTOSCELIS TRICOLOR (HEMIPTERA: COREIDAE)

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MATERNAL EFFECTS AND SEXUAL SELECTION IN THE HELICONIA BUG,

LEPTOSCELIS TRICOLOR (HEMIPTERA: COREIDAE)

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Maternal Effects and Sexual Selection in the Heliconia Bug, *Leptoscelis tricolor*

(Heteroptera: Coreidae)

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   The early environment experienced by organisms can have significant phenotypic effects that persist throughout life. Parents, mothers in particular, commonly determine these early environments. Maternal effects, or the influence of maternal phenotypes on offspring phenotypes, are therefore likely ubiquitous in natural populations. However, the frequency and evolutionary consequences of maternal effects are largely unknown.

   Here I show that maternal phenotypes can have large effects on offspring morphology, fecundity, and behavior. I theoretically explore the potential for these effects to resolve the long-standing "lek paradox" in the field of sexual selection. I also empirically investigate these effects in the heliconia bug, *Leptoscelis tricolor*.

   Females heliconia bugs lay eggs on several species of heliconia plants in Panama and Costa Rica. Host plant species choice by mothers largely determines the natal environment that offspring will experience due to the limited mobility of juveniles. Insects that emerged on one species of heliconia, *Heliconia platystachys*, were larger, and males expressed relatively larger secondary-sexual traits for their body size than those raised on *H. mariae*.   The mating probability and fecundity of female offspring was higher on *H. platystachys*. Furthermore, males raised on *H. platystachys* performed a faster rate of copulatory courtship while mating and also boosted this rate when mating with females from *H. platystachys*. Such copulatory movements may enhance male and female reproductive success.

   While host plant species choice by mothers had significant maternal effects on offspring overall, the consequences for offspring varied with time. Mothers that laid eggs on *H. platystachys* early in the wet season produced large, fecund offspring in good phenotypic condition. This maternal effect gradually changed until, later in the season, sons and daughters raised on *H. platystachys* were smaller and daughters laid few or no eggs and did not mate. Thus, at some times *H. platystachys* appeared to be a much superior host plant for offspring, while at other times, *H. mariae* was better. Dynamic consequences of maternal behaviors (here, host plant species choice) for offspring have only rarely been explored and may have far-reaching consequences for the evolution of maternal and offspring traits.

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CHAPTER 1

INTRODUCTION

Phenotypic variation provides the raw material for natural selection and adaptive evolutionary change. Understanding the factors that contribute to phenotypic variation is therefore necessary for predicting evolutionary trajectories. Much current and past research has focused on direct genetic influences on phenotypic variation. The genes of individuals are clearly important in trait expression, however environmental factors can have notable and even overwhelming influences on the expression of many traits in natural situations. Environmental factors can alter the rate and even direction of the response to selection pressures, and thus complicate our understanding of the evolutionary process.

Maternal effects, or the influence of a mother's phenotype on the phenotypes of offspring, have recently become recognized as important environmental influences on phenotypic variation in offspring. Mothers often influence offspring both pre-natally and post-natally through provisioning seeds and eggs with nutrients and hormones, selecting offspring habitat, and providing parental care. Even though maternal effects are likely ubiquitous in natural populations, the frequency and consequences of maternal effects in natural populations are virtually unknown.

For my dissertation research, I empirically explored maternal effects on offspring morphology and behavior in the heliconia bug, *Leptoscelis tricolor*. I also theoretically addressed the potential for maternal effects to help resolve the long-standing "lek paradox" in the field of sexual selection. The following chapters focus on maternal effects, but paternal effects can be considered in an analogous way.

I devoted my field studies to heliconia bugs, a relatively unknown species of insect. I spent several months early in my graduate career conducting natural history observations of this insect in Panama and Costa Rica (see also Chapter 2). I learned that this insect uses several species of heliconia plants for oviposition, and that offspring nearly always remain on the host plant species where mothers lay eggs. Therefore, the host plant choice of mothers largely determines the environment where offspring grow

and develop. By the time that offspring develop wings and can fly away to select their own host plant, their body dimensions have been determined for life.

The size and shape of insects often have large effects on their reproductive success and fitness. In heliconia bugs, males use their hind legs in competitions with other males over heliconia inflorescences. Successful males gain territories on these inflorescences and commonly mate with females feeding on the plants. Size and shape are not only probably important to male heliconia bugs, but also to females; female size has significant consequences for egg production.

I describe my observations of the natural history of the heliconia bugs in Chapter 2, focusing on behavior, distribution, movement, oviposition, and parasitism. I also compare these characteristics of heliconia bugs with characteristics of other coreid species.

In Chapter 3, I explore the influence of host plant species choice by mothers on the expression of hind legs and other body size and shape characteristics in heliconia bugs. I found that mothers can have large influences on the morphology of offspring, simply by selecting one host plant species over another for oviposition. Furthermore, this maternal decision has different implications for offspring at different times of the year. These differences appear to be a result of phenological changes in the host plant species. Context-dependent maternal effects, such as I demonstrate in Chapter 3, have been virtually unexplored.

The role of host plant species in determining insect morphology has implications for our understanding of condition-dependent expression of secondary-sexual traits. Secondary-sexual traits are expected to evolve a heightened sensitivity to the condition of an individual, so that animals in good condition will be able to express larger or more elaborate traits. These traits thus become accurate indicators of condition and may be used by conspecifics in mate choice or competitive decision making.

Many empirical studies have recently tested whether secondary-sexual traits exhibit condition dependence as predicted by theoretical models. Results have been mixed, perhaps due to the common use of highly-artificial laboratory environments to assign condition to groups of individuals. Therefore, studies involving natural alternate environments are a necessary complement to laboratory studies. In Chapter 4 I discussed

the potential importance of field studies to this body of work, and I examine patterns of trait expression in heliconia bugs raised on different host plant species. I found that the size of male hind legs was influenced by natal host plant environment, and that there were significant differences in leg size *relative to body size* for insects raised on alternate plant species. Other male and female morphological traits did not exhibit similar differences in relative size. These patterns suggest that male hind legs are not normal, metric traits that scale with body size, but instead are highly sensitive indicators of male condition.

The selection of host plant species by mothers may commonly have effects that go beyond offspring morphology. In Chapter 5, I explore the influence of host plant species choice by mothers on offspring mating behaviors and fecundity. I found that maternal host plant species choice affected the performance of copulatory courtship behaviors by males, female mating probability, and female fecundity. However, these effects were not consistent over time. As with offspring morphology (Chapter 3), maternal host plant choice had dynamic influences on offspring mating behaviors and fecundity over time, likely due to predictable changes in the environments provided by host plant species. The role of maternal behaviors and natural environmental change in determining offspring reproductive traits suggest that an individual's genetic quality may commonly play a minor role in reproductive success.

The historic lack of attention given to maternal effects by most evolutionary biologists may be due to the misperception that maternal effects will have little or no effect on trait evolution. However, maternal effects commonly have a genetic basis in mothers. Therefore, genes responsible for trait variation in offspring may be in *the previous generation*. Such indirect genetic effects may accelerate, slow, or even reverse the evolutionary response to selection pressure. The ubiquity of maternal effects in natural populations combined with their potential complicating effects on evolutionary dynamics warrants greater empirical and theoretical attention than currently given.

In Chapter 6, I argue that maternal effects may help resolve the "lek paradox." When maternal effects on male secondary-sexual traits are present, females choosing to mate with males with elaborate secondary-sexual traits may receive heretofore-unexplored genetic benefits for offspring. Males expressing elaborate traits may have

received good parenting (e.g. good host plant choice in the heliconia bugs; Chapter 3), and when this good parenting has a genetic basis in mothers, these genes can be passed on across generations, so that elaborate males may pass "good *parenting* genes" onto their progeny.

CHAPTER 2

THE NATURAL HISTORY OF THE HELICONIA BUG, *LEPTOSCELIS TRICOLOR*

(WESTWOOD)

<u>Introduction to the life history, morphology and behavior</u>

My studies of the heliconia bug, *Leptoscelis tricolor*, were conducted in Panama and Costa Rica during the wet season in the years 2002-2005, with a focus on the months of June through October.

Leptoscelis tricolor relies upon the inflorescences of heliconia plants for all stages of its life history. Nymphs complete five instars on heliconia plants, commonly hiding within inflorescence bracts and emerging to feed on the developing fruits, nectar from flowers, and phloem of the inflorescences. Adults also use these food sources, and compete, mate and oviposit on inflorescences. Adults can live at least 80 days or more in protected conditions (see Chapter 5). The generation time of heliconia bugs is approximately 30 days, likely varying with temperature and food availability as in other coreid species (e.g. Carroll, 1980; Mitchell, 2000). Eggs hatch after approximately 14 days. The first instar nymphs do not feed, but do require access to water. The first nymphal stage last approximately 2 days. The second, third, fourth, and fifth nymphal stages are all approximately 5 to 10 days long. The exoskeleton of new adults remains soft for over 2 days, and reproductive maturity can be achieved within 10 days of molting. However, in some individuals experiencing poor conditions as nymphs, reproductive maturity may be delayed (see Chapter 5).

Both male and female heliconia bugs have enlarged hind femurs with spines, though the size of male legs is visibly larger than those of females. When males encounter one another, one insect will commonly begin twitching his front legs up and down. The other male may run away, or may, in turn, begin twitching his front legs up and down. I have also witnessed males flattening themselves out completely on a heliconia inflorescence when approached by a twitching male. This behavior may be

most common when males are competing for a feeding site; the flattened male will continue to feed while the other male climbs on top of him and appears to be trying to remove him from the plant. Usually, once males were flattened out, they could not be removed by other males. However, in one instance, I did observe a male peel the other male completely off the plant so that he dropped below.

If a male responds to the leg twitches of another male by twitching his own legs up and down, an escalated competition ensues. Males will turn around and carefully wrap their hind legs around each other. When the spines of their hind femurs are aligned with the abdomen of their rival, the males squeeze with their hind legs using intense bursts. This squeezing may continue for some time, with the males falling of the plant and continuing their competition below. Eventually, one male will return to the inflorescence while the other leaves the area. Because of the complex, three-dimensional environment of heliconia plants, the competitions of male heliconia bugs may not be stereotyped as described above. In some situations I have witnessed males having difficulty wrapping their hind legs around each other, with one hooking another's antennae or dragging the other male by a leg.   On two occasions over my ~ 16 months in Central America I witnessed females competing in the way described above for males. Competitions are generally rarely witnessed in the field, either between males or between females. Most of my observations of competitions were conducted indoors under experimental conditions.

Courtship, on the other hand, was commonly observed both in the field and in the laboratory (see also Chapter 5). Reproductively mature, unmated females will approach males, sometimes waving their mouthparts and legs. Females that have previously mated often appear to ignore males, though they frequently will mate on multiple occasions. Courtship of previously-mated females appears to be longer in duration than courtship of sexually-naïve females.   When males encounter females, they commonly begin twitching their front legs up and down (a similar behavior as males perform when initiating a competition with another male). A male will then begin tapping a female with his front legs. At this point, females will remain motionless, kick at the male, or move away. If a

female remains motionless, a male will then climb upon her, facing the same direction she faces, hang off her sideways, and initiate copulation. Once copulation has begun, the male pivots outward, so the insects are both facing opposite directions. Females nearly always feed while mating (Figure 1) in heliconia bugs and other coreids (CW Miller, *personal observation*), often using the same feeding spot as they were before mating was initiated. Interestingly, insects will compete for feeding sites in this species, and males may withhold access to feeding sites from females that will not copulate.

Copulations commonly last for several hours or more, and appear as likely to occur during the day or night. During copulations, males commonly perform copulatory courtship (*sensu* Eberhard, 1994). Males will rock back and forth, then stroke the abdomen of females with their hind legs. I have found a correlation between the rate of copulatory courtship performed by males and the number of eggs laid immediately after mating by females (Chapter 5).

Female and male heliconia bugs often mate multiply over their lifetimes, as is common in many coreids. Such repeated matings may allow for cryptic female choice. Sperm limitation is an unlikely proximate cause of the large number of rematings that occur in many species of coreids. In the golden egg bug, *Phyllomorpha laciniata,* females mate often but can lay fertile eggs for more than a month after a single copulation (Katvala & Kaitala, 2003). The mating system of coreids varies from species to species and includes scramble competition polygyny (Nishida, 1989), female defense polygyny (Fujisaki, 1981; Nishida, 1989), and resource defense polygyny (Mitchell, 1980).

Figure 1. A pair of *Leptoscelis tricolor* adults in copula. Note that the female (foreground) is feeding.

Oviposition, movement and feeding

Females lay their eggs singly, and because of movement patterns, they may lay eggs on multiple inflorescences over their lifetimes. Both males and females frequently move from inflorescence to inflorescence; they seldom spend more than a few days at any one inflorescence. In 2005 a research assistant marked 259 insects on Barro Colorado Island where heliconia plants are common along the edges of the laboratory clearings. Of the 79 insects he resighted, 92% were found on different heliconia inflorescences than when they were first sighted. 33% of insects resighted were on different heliconia species. The percentage was similar for both males and females. Because many insects likely left the study area or were overlooked during surveys, the number of insects switching host plant species over their lifetimes was likely much higher for this population. Other populations would likely vary in the number of insects switching host plants due to the diversity, distribution, and abundance of heliconia plants in the area.

Because the different species of heliconia host plants have significant effects on the behavior and morphology of offspring (Chapters 3-5), I attempted several methods to evaluate the oviposition preferences of female heliconia bugs. In the wild, females likely exhibit oviposition preferences at a variety of scales: long distance orientation and host searching, as well as discriminating at shorter distances. Thus, the best assessment of oviposition preferences would measure female behavior at several scales; a challenging endeavor at best.

I measured close-range female oviposition preferences placing 27 pairs of heliconia bugs on either *Heliconia mariae* or *H. platystachys* and counting the number of eggs laid after two weeks. In designing this no-choice experiment, I selected approximately equal numbers of females and males that had been reared on *H. mariae* or *H. platystachys* and emerged in early August, 2005. I divided the pairs equally according to natal host plants across *H. mariae* and *H. platystachys* inflorescences, in a stratified-random design. I left these pairs in bags on the inflorescences for two weeks between

September 1st and 14th, 2005. I then brought the bag and inflorescence into the laboratory to count eggs. I found that females placed on *H. platystachys* produced significantly more eggs than those placed on *H. mariae* ($F_{1,21} = 4.19$, $p = 0.05$; Figure 2). Neither the natal host plant of females nor of males had no significant effect on the number of eggs produced (Females: $F_{1,21} = 1.04$, $p > 0.30$; Males: $F_{1,21} = 1.08$, $p > 0.30$).

These results are intriguing, given the poor performance of newly-emerged adults on *H. platystachys* in October (see Chapters 3-5). Eggs laid in early September will become the "October cohort" described in these studies. However, this mismatch between oviposition preference and offspring performance is not an anomaly; in many species, females do not show signs of preferring the host plant where offspring will perform best (Mayhew, 2001; Thompson, 1988; Valladares & Lawton, 1991; Mayhew, 1997). In this case, females on *H. platystachys* may have simply benefited from better nutrition, and as a result, produced more eggs.
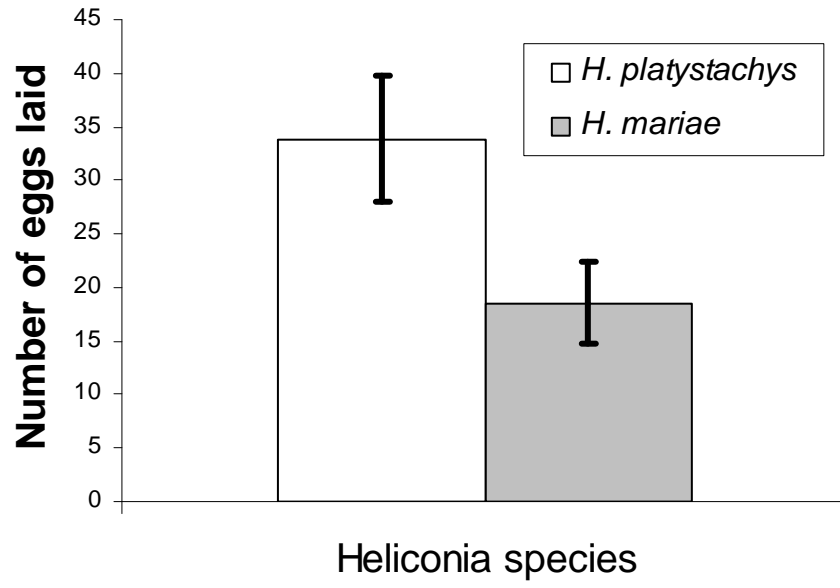
Figure 2. Egg production by females on *Heliconia platystachys* or *H. mariae* over a two week time period in early September 2005.

In addition to the no-choice, population-level study of oviposition preferences described above, I also attempted a variety of other assays of oviposition preferences. However, these other studies were not as productive due to the oviposition behavior of this species. In one case, I conducted simultaneous-choice tests with multiple inflorescences presented to a female in a 1m x 1m x 1m cage. Through this work and observations of wild females, I discovered that female heliconia bugs will not only lay eggs on inflorescences, but also nearby. The lack of oviposition preferences in a simultaneous-choice setting may reflect this tendency and also the walking ability of nymphs. Nymphs in the wild commonly walk across vines and leaves to nearby inflorescences. This dispersal is limited, but makes conducting a simultaneous-choice experiment spatially difficult. The challenges of laboratory assessments of oviposition preference as well as the difficulty of finding the small, brown eggs in the field, may limit future studies of oviposition behaviors in this species.

I have found nymph heliconia bugs on all heliconia species that I have surveyed in abundance in Costa Rica and Panama: *H. platystachys, H. mariae, H. latispatha, H. irrasa, H. pogonantha, H. imbricata, and H. hirsuta*. I found no nymphs on the exotic, small heliconia ornamental (possibly a cultivar of *H. psittacorum*) which is commonly found in gardens and landscaping in the Gamboa, Panama area. Many of the heliconia species used by heliconia bugs do not produce heliconia inflorescences year-round (see Chapter 5). Thus, these insects must 1) leave patches of heliconia when no viable inflorescences remain and seek out different patches of heliconia as new inflorescences become available and/or 2) become dormant when no viable inflorescences remain in a patch and wait until the next season. My data above shows that insects do switch heliconia host plant species at least during the months I surveyed, but dormancy is a possible strategy that some individuals may use.

Leptoscelis tricolor and other Coreidae have piercing-sucking mouthparts. I have witnessed two different feeding behaviors in *Leptoscelis tricolor.* When feeding directly through the bract face or on flowers, these insects remain motionless, while up and down movement is common while feeding on fruits. These two different feeding behaviors

may indicate that these insects may be using a combination of osmotic pump, lacerate-and-flush, or macerate-and flush feeding strategies (see also Mitchell, 2006). The use of more than one feeding strategy in a single coreid species has not been documented, though there is evidence that some species of coreids use different strategies than other species in this same family (Mitchell 2007). Further study of modes of feeding in this insect family may yield insights into the evolution of generalist feeding strategies (Mitchell, 2006).

I have commonly witnessed the reuse of feeding holes in adult and nymph heliconia bugs. In addition, adult bugs also commonly defend feeding holes. I recall several occasions where nymphs appeared to be waiting for adults to finish feeding so that they could use the same feeding holes. I also have seen nymphs sharing feeding holes with adults, however adults rarely allowed this to occur and would kick away intruding nymphs.

Among coreids, the apparent value of feeding holes appears at first glance to correlate with the toughness of the plant material. For instance, I have not observed reuse of feeding holes in squash bugs (*Anasa tristis*, *Anasa andresii*, and *Anasa scorbutica*) feeding on squash plants and cactus bugs (*Chelinidea vittiger* and *Narnia femorata*) feeding on *Opuntia* cacti. However, reuse of feeding holes may be common in coreids feeding on tree trunks (such as *Acanthocephala declivis*; Eberhard, 1998).

<u>Coloration and crypsis in heliconia bugs</u>

The inflorescences of different heliconia species may include greens, yellows, oranges, pinks, reds, and even blacks (due to dead and wet plant material). To the human eye, *Leptoscelis tricolor* adults are perfectly camouflaged against *Heliconia mariae's* red and black inflorescences. However, these same bugs stand out against the lighter *Heliconia platystachys* and *Heliconia latispatha* inflorescences. This striking difference in the degree of crypsis suggests that *Heliconia mariae* may have been a more common host plant in the evolutionary past.

Nymph heliconia bugs are black and red for their first three stages of life (Figure 3A). Beginning in the fourth instar, nymph color may vary in saturation along a continuum. By the fifth stage, these differences are even more apparent (Figure 3B).

I hypothesized that this variation in coloration is due to phenotypic plasticity, with nymph expressing coloration that enhances crypsis against their background. To test this hypothesis, I conducted a split-brood breeding experiment and natural observations (C.W. Miller, *in preparation*). My preliminary analysis using the subjective scores of "dark," "medium," or "light" to describe nymphs from the split-brood breeding experiment found that 100% of 5th stage nymphs (n = 8) successfully reared on *Heliconia mariae* were dark. 62% of those successfully reared (n = 13) on *Heliconia platystachys* were dark, and only 30% of those successfully reared (n = 37) on *Heliconia latispatha* were dark. These preliminary data suggest that color may be phenotypically plastic in *Leptoscelis tricolor* nymphs. Alternatively, the differences in color seen in 5th stage nymphs may reflect selective mortality of nymphs according to coloration on the different plant species.

Figure 3. Juvenile heliconia bugs. In (A) a young *Leptoscelis tricolor* nymph on *Heliconia mariae*. Nymphs in the first few instars do not visibly differ in coloration from one another. In (B) 5th instar nymphs of *Leptoscelis tricolor*. The "light" form (right) is only found on *Heliconia platystachys* and *H. latispatha* plants. The "dark" form (left) may be found on *H. platystachys* and *H. latispatha* plants, but is most commonly associated with *H. mariae* plants.

Congeners in Panama and Costa Rica

The genus *Leptoscelis* contains at least three species: *L. tricolor, L. quadrisignata,* and an unidentified species (*Leptoscelis* sp 1) found in Golfito, Costa Rica and other locations (*INBIO* insect collection, San Jose; CW Miller, *personal observation*; Figure 4). In the Gamboa area of Panama, I commonly observed *L. quadrisignata* on *Calathea* sp., especially *Calathea latifolia* (Figure 5).

Figure 4. *Leptoscelis* sp1 in the INBIO collection, Costa Rica. Note that the male (left) has enlarged hind femurs with prominent spines relative to the female (right).
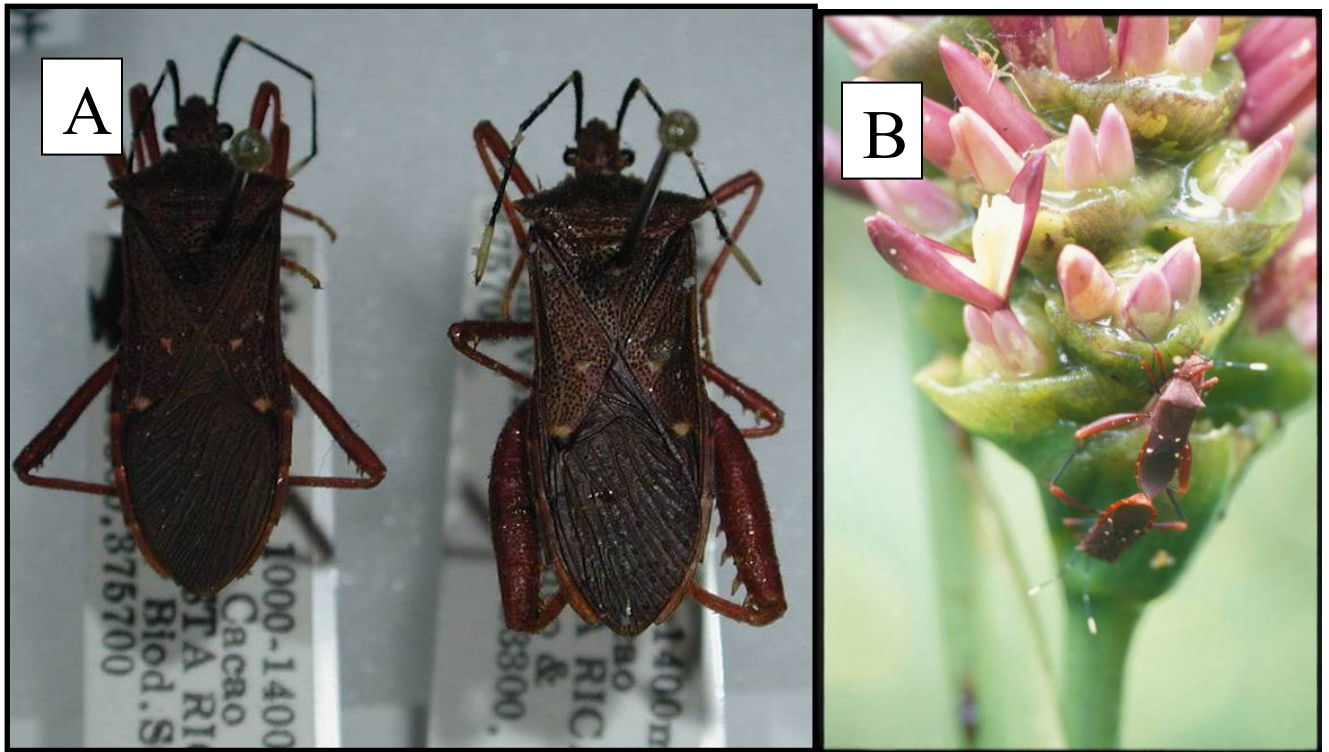
Figure 5. *Leptoscelis quadrisignata.* In (A) specimens from the INBIO collection in Costa Rica are pictured. Note that the male (right) has enlarged hind femurs with prominent spines relative to the female (left). In (B) *Leptoscelis quadrisignata* mating on *Calathea latifolia* on Pipeline Road near Gamboa, Panama.

New host record for *Hexacladia* near *townsendi*

While studying *Leptoscelis tricolor* I occasionally noticed adults or 5th instar nymphs with holes in their thorax and abdomen. One captive nymph remained in the 5th instar for 20 days, ten days longer than the other insects reared in similar conditions. During this time, the abdomen darkened and looked unusual. Ten parasitoids (nine females and one male) eventually exited through four dorsal and ventral holes in the abdomen of the individual (Figure 6). The nymph remained alive for a few more days, walking around with holes, allowing light to pass through the abdomen (Figure 7).

The parasitoid species is very close to *Hexacladia townsendi* (Crawford) which was described as a parasitoid of *Stenomacra* species in Peru (John Noyes, *personal communication*). This species is in the family Encyrtidae and order Hymenoptera.
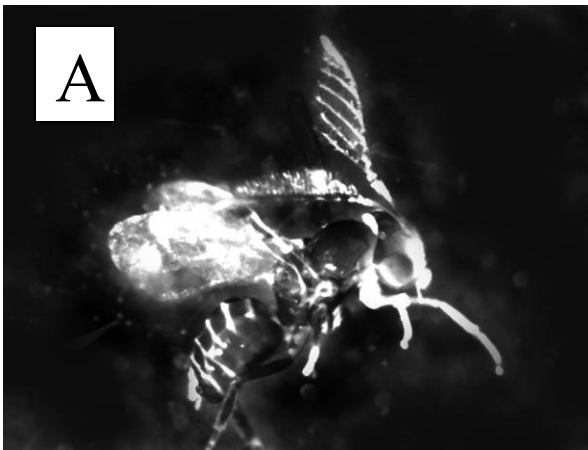
Figure 6. *Hexacladia* near *townsendi* parasitoids that emerged from a *Leptoscelis tricolor* nymph. In (A) the male, and in (B) one of the nine females that emerged. Insect identification by John Noyes at the Natural History Museum, London.

Figure 7. Dorsal view of the 5th instar nymph from which six parasitoids emerged. Note the abdominal holes with melanized rims from which the parasitoids exited.

The distribution patterns of *Leptoscelis tricolor*

Over the course of my studies of the heliconia bug, *Leptoscelis tricolor*, I realized that the availability and phenology of heliconia inflorescences of different species changes dramatically over time (Stiles, 1975; Chapter 5). With the likely variation in resource quality, I hypothesized that the distribution patterns of these insects would vary over time across the species of host plants.

I examined the distribution patterns of heliconia bugs across three common heliconia species in the Gamboa area: *Heliconia platystachys, H. mariae, and H. latispatha.* My assistants and I looked for adult and 5th stage nymph heliconia bugs on heliconia inflorescences between the dates of August 24th and August 31st, 2005 and again between September 24th and October 1st, 2005. We surveyed a total of 560 *Heliconia platystachys* inflorescences (226 and 334, for each survey period, respectively), 3196 *H. latispatha* inflorescences (1384 and 1812, respectively) and 641 *H. mariae* inflorescences (409 and 232, respectively). Our survey procedures were as follows: first, we approached an inflorescence haphazardly, looking at each side. Next, we looked within each bract for nymphs and adults. I constructed logistic regression models to examine the effect of heliconia species, time period of sampling, and the interaction on the presence of adults or 5th stage nymphs on inflorescences. I also examined whether nymphs were more likely to be found with other nymphs (of any nymphal stage) on some plant species and time periods versus others.

I found that these three heliconia species differed significantly in the probability that adults or nymphs would be present (Table 1; Figure 8). The time period of sampling did not influence the probability of finding adults or of finding adults on certain heliconia species. The probability of 5th stage nymphs being present, on the other hand, did vary according to the time of sampling and the interaction between heliconia species and time (Figure 8b). Most notably, we had a much higher probability of finding nymphs on *H. platystachys* in the earlier sampling period than the latter. The probability of finding nymphs on *H. mariae* actually increased later in the season. These results suggest that

either females laid fewer eggs in *H. platystachys* later in the season or that nymphal mortality increased on this host plant. The study of female oviposition preferences (above) showed that females laid *more* eggs on *H. platystachys* than *H. mariae* in early to mid-September 2005. This study supports the hypothesis that nymphal mortality is responsible for the low numbers of on *H. platystachys*. However, further study is needed. These results are intriguing in context of the temporal changes in host plants and insect phenotypes as is described in Chapters 3 through 5.

I found that 5th instar nymphs on *H. latispatha* were significantly less likely than those on other species of heliconia to be found with other nymphs. In fact, only 14 *H. latispatha* inflorescences out of 3196 had more than one nymph present. This contrasts with 42 of 560 *H. platystachys* and 41 of 641 *H. mariae* inflorescences. This result may reflect the smaller size and fewer hiding spots on *H. latispatha* inflorescences as compared to the other species.

Table 1. Results of logistic regressions for the probability that insects were present on a heliconia inflorescence

Source	adult(s)		nymph(s)	
	d.f.	χ^2	*d.f.*	χ^2
Heliconia species	2	31.46***	1	101.29***
Time of sampling	1	2.41	1	20.79***
Heliconia x time	2	4.73	1	25.13***

* P < 0.05, ** P < 0.02, *** P < 0.001.

Table 2. Results of logistic regressions for the probability that nymphs would be found in groups rather than singly.

Source	adult(s)	
	d.f.	χ^2
Heliconia species	2	8.18**
Time of sampling	1	0.63
Heliconia x time	2	3.70

* P < 0.05, ** P < 0.02, *** P < 0.001.
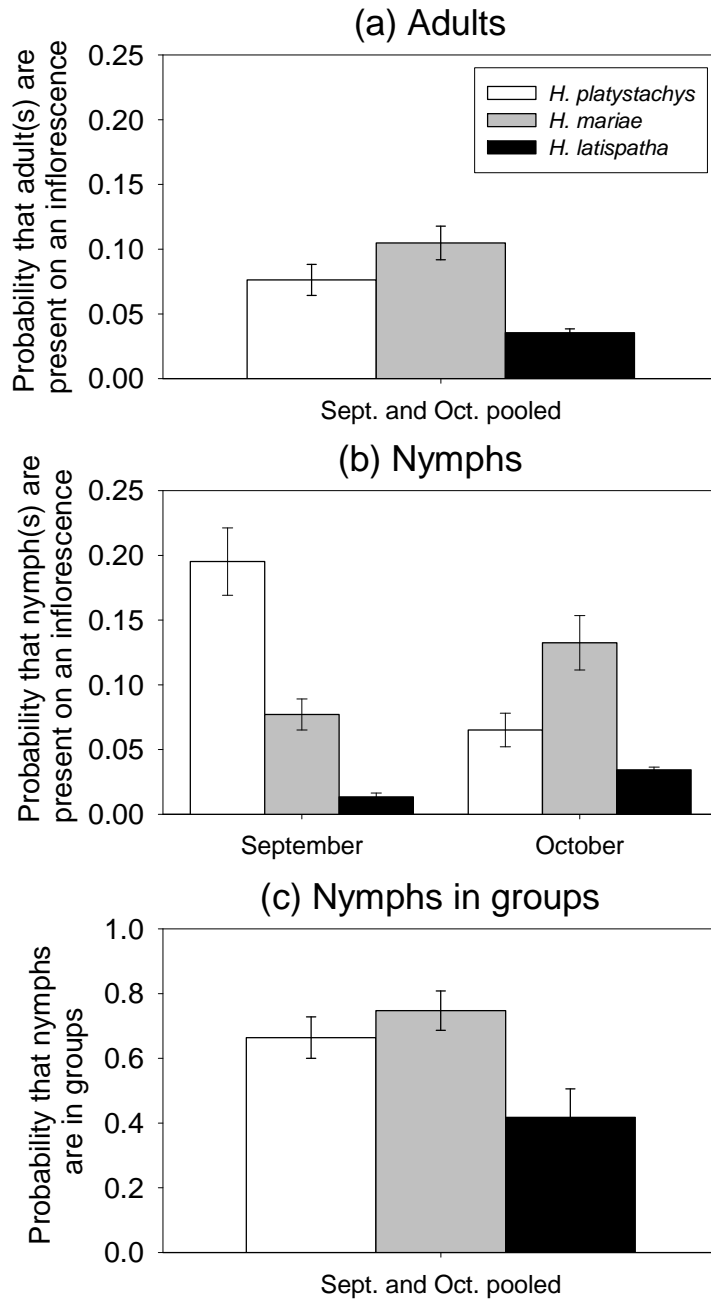
Figure 8. The probability that a single heliconia inflorescence will have (a) adult(s) or (b) nymphs of *Leptoscelis tricolor* present. If a nymph was located on an inflorescence (c) examines the probability that the nymph was found with other nymphs. In (a) and (c) the two survey periods were not significantly different and were therefore pooled for graphical purposes.

In many species of coreids, either the nymphs, adults, or both are commonly found in aggregations. They often do not use all the resources available in an area, and instead concentrate on just a few individual plants. Some examples of species with aggregating adults include *Colpula lativentris* (Nishida, 1989), *Acanthocephala declivis* (Eberhard, 1998) *Leptoglossus australis* (Yasuda, 1998), and *L. occidentalis* (Blatt & Borden, 1996). Generally, these aggregations appear to be related to mating activities, though in *Leptoglossus occidentalis*, adults aggregate when overwintering (Blatt & Borden, 1996). Aggregation and/or sexual pheromones are suspected to be used in initiating these aggregations (Yasuda, 1998; Wang & Millar, 2000).

Aggregation behavior is common in nymphs of many coreid species, including *Thasus acuntangulus* (Aldrich & Blum, 1978), *Chelinidea vittiger* (DeVol, 1969), *Narnia femorata* (CW Miller, *personal observation*), and *Anasa tristis* (CW Miller, *personal observation*). Nymphal aggregations may be due to collective use of a few feeding holes (see above), for combined aposematic defense, or for other reasons. Some coreid nymphs are brightly colored and release alarm pheromones when disturbed, suggesting combined aposematic defense (Aldrich & Blum, 1978). However, other species are more cryptic and are less likely to release alarm pheromones (such as *Chelinidea vittiger*). In this species, nymphs commonly aggregate, with each facing outward from a central point, suggesting collective vigilance may be a benefit of aggregating. *Leptoscelis tricolor* nymphs are edible to birds (Miller *et al., in preparation*) and often appear more cryptic than aposematic (depending upon host plant species). Thus, they might also benefit from aggregative behaviors in the same manner as might *Chelinidea vittiger* nymphs.

Given the apparent ubiquity of aggregative behaviors in this family of insects, I hypothesized that *Leptoscelis tricolor* adults and nymphs would also aggregate. However, according to my distribution patterns, there is no evidence for the presence of aggregations in either adults or in nymphs (CW Miller, *unpublished data*).

Alarm pheromones are commonly used in the Coreidae and likely serve to deter predators and also function as an alarm signal to insects nearby (Aldrich & Blum, 1978; Blatt & Borden, 1996; Eberhard, 1998; Hartwig & De Lange, 1978; Mitchell, 2000). Volatiles include compounds such as acids, aldehydes, alcohols, and acetate or butyrate esters of these alcohols (reviewed in Mitchell, 2000). When disturbed, adult heliconia bugs commonly emit an alarm pheromone that smells like green apply candy. Other nymphs and adults are highly sensitive to this alarm pheromone and quickly hide or fly away after it has been emitted. Interestingly, alarm pheromones emitted by one species of coreid may be used by other species (Leal et al., 1994; CW Miller, *personal observation*). Many coreids, including *Leptoscelis tricolor*, also emit anal fluid when disturbed. Some coreids direct streams of anal fluid towards potential predators (e.g. Hingston, 1929; Aldrich & Blum, 1978).

Morphology of wild-caught adults

The sizes of newly-emerged adults vary significantly by host plant species and the time of emergence (see Chapter 3). However, my movement data suggests that adults commonly change host plant species during their lifetimes (see above). Thus, the sizes of mature adults found visiting different plant species may be different than the sizes of new adults emerging on those same plants. The range and variation in adult sizes may have implications for a variety of ecological and evolutionary phenomena including the intensity of natural and sexual selection and also may reflect differences in the ability of different-sized individuals to disperse. Thus, this foundational data may be useful for future studies with this species.

I measured wild-caught, mature adults for pronotum width (a common metric of body size in insects), the width of both hind femurs, head length, and left front femur length. I did not include soft adults in this study, because they were almost assuredly young and reproductively-immature. Measurements were conducted in the same manner as for other studies (see Chapters 3 and 4). I marked all adults before releasing them, to ensure that they were not measured again on a different occasion. We had difficultly

locating adults on *H. platystachys* in September, and as a result, no males were measured during that sampling period.

I found that mature adults captured on *H. platystachys* and *H. latispatha* were generally larger than those mature adults found on *H. mariae* (Table 3; Figure 9). The host plant effect varied for females over time (Table 3; Figure 9). These size differences across host plants may be driven by the slow dispersal of adult heliconia bugs; adult heliconia bugs are relatively poor fliers. These bugs often fly short distances, land on a leaf or inflorescence, reorient, and fly another short distance. New adults emerging on *H. platystachys* (Chapters 3, 4) and *H. latispatha* (CW Miller, *unpublished data*) are larger than those emerging on *H. mariae* at certain times of the year. Interestingly, the patterns of mature adult female sizes do appear to mirror what I found for newly emerged adult females (Chapter 3), except for in the month of July. This disparity for July may reflect the dispersal of mature females *into* patches of *H. platystachys* from other heliconia species (likely *H. mariae*) and the tendency for females to stay within these patches while the inflorescences are still available. *H. platystachys* does not produce viable resources for heliconia bugs much the year, so it may have to be colonized anew each year (generally May-July) when new inflorescences are produced (see also Chapter 5). The flood of large, newly-emerged adults produced on *H. platystachys* may then account for the boost in size of mature adults captured during the months of August through October. Further work is needed to explore the fluctuating sizes of heliconia bugs over time and host plants, and what these patterns might mean to the ecology and evolution of this species.

Table 3. Results of analysis of variance for body size traits
of mature adults

source	d.f.	Pronotum width		Hind femur width	
		Males	Females	Males	Females
		F	F	F	F
plant species	2	4.28**	8.16**	5.47**	2.08
sampling period	3	2.60	1.10	2.47	2.51
plant x sampling period	5	0.39	2.39*	0.19	2.40*

Error df = 123 (males), 75 (females)

*P < 0.05, ** P < 0.02, *** P < 0.001.*
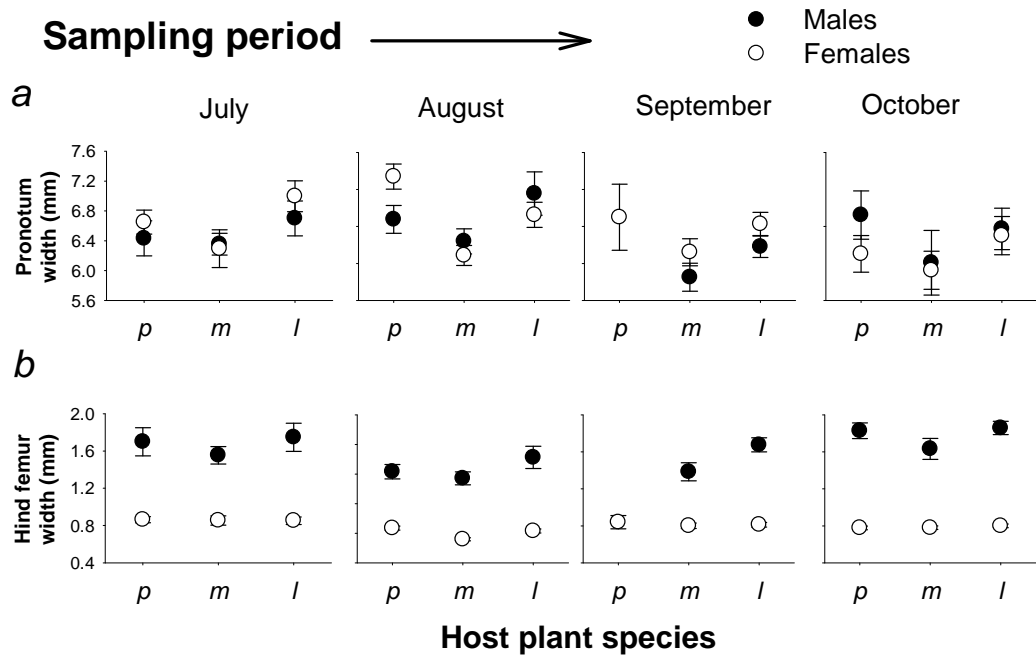
Figure 9. The pronotum width (a) and mean hind femur width (b) for mature, adult males (solid circles) and females (hollow circles) found on three different heliconia species during each of the sampling periods (least-squares means $\pm$ standard errors). The labels "p", "m", and "l" represent *Heliconia platystachys, H. mariae,* and *H. latispatha* respectively.

The allometric slope between femur width and body size (pronotum width) differs by sex (Chapters 3, 4). Therefore, I tested for allometric intercept differences (i.e. trait size relative to body size) of hind femur width separately for males and females. The allometric intercept of hind femurs (i.e. hind femur width relative to body size) was *not* significantly different for males captured on different species of heliconia (Table 4; Figure 10). This contrasts with the significant effect of *natal* host plant species on newly-emerged adults for some cohorts of insects (Chapters 3, 4). While the allometric intercept of female hind femurs *was not* significantly different *overall* for females captured on different species of heliconia, the allometric intercept of hind femurs did fluctuate significantly with sampling period (Table 4; Figure 10). In addition, the effect of plant species on the allometric intercept of female legs changed significantly over the sampling periods. These differences are largely driven by the females captured on *H. platystachys* and *H. mariae* in July (Figure 10). Why the allometric relationship between body size and leg size in females should vary, and vary in this manner, is currently a puzzle and warrants future research.

Table 4. Results of analysis of covariance for the allometric intercept of hind femur width with body size (pronotum width) as a covariate. The analyses were performed separately for males and females.

source	Males		Females	
	d.f.	F	d.f.	F
body size (pw)	1	461.86***	1	107.07***
plant species	1	2.80	2	1.56
Sampling period	3	2.54	3	7.70***
plant x sampling period	6	2.54	6	2.38*

Error df: Males = 124; Females =74

*P < 0.05, ** P < 0.02, *** P < 0.001.*

a) Males
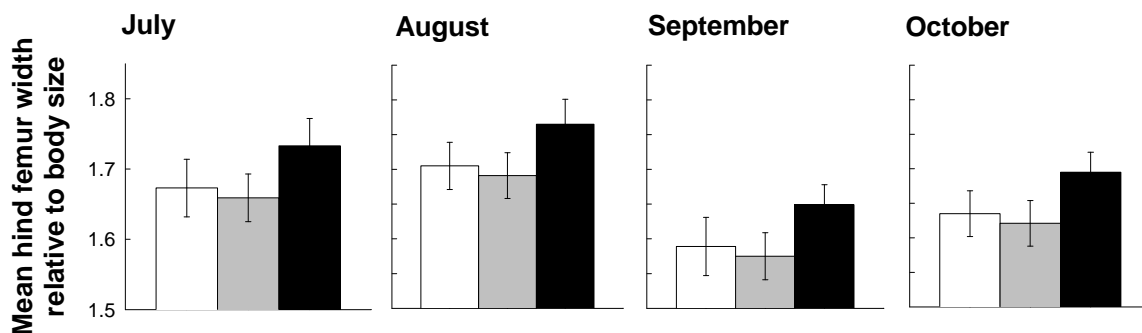
b) Females
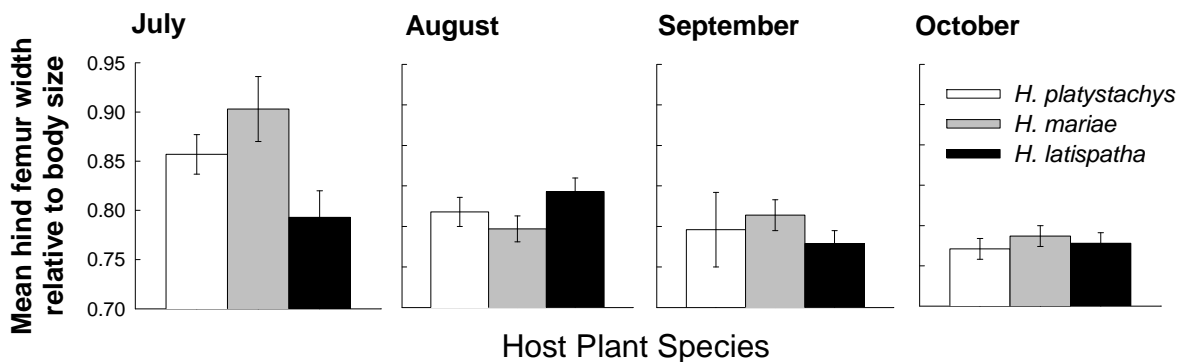
Figure 10. Relative hind femur width for mature, adult males (a) and females (b), found on three heliconia species over four sampling periods (least-squares means $\pm$ standard errors).

CHAPTER 3
MATERNAL EFFECTS ON TRAIT SIZE AND ALLOMETRIES IN CHANGING
NATURAL ENVIRONMENTS

Abstract

Mothers can influence the phenotypes of their offspring by providing care, provisioning food, and selecting offspring habitat. Importantly, such maternal effects do not occur in a vacuum, and they can be modified by ambient environmental conditions. However, few studies have explored the effects of natural environmental variation on the magnitude and direction of maternal effects on offspring. We investigated the influence of host plant species choice by mothers on the expression of offspring morphological traits in the heliconia bug, *Leptoscelis tricolor* (Heteroptera: Coreidae). We repeated this study on four sequential cohorts of offspring to determine whether the relationship between maternal behavior (host plant species choice) and offspring phenotypes was modified by seasonal changes in host plant environments. We found that host plant species choice by mothers had significant effects on the expression of morphological traits including male secondary-sexual traits and the level of sexual dimorphism. Moreover, seasonal changes in host plant phenology substantially modified the expression and magnitude of the maternal effects on offspring. Our results suggest that understanding of the ecological and evolutionary roles of maternal effects may be vastly improved by measuring them in multiple natural contexts.

Introduction

Understanding the causes of phenotypic variation is of vital importance to evolutionary ecology because variation provides the raw material for natural and sexual selection. Parents, mothers in particular, often influence offspring phenotypes by providing care, provisioning food, and selecting offspring habitats. In many species such maternal effects on offspring can be crucial for early growth and development and can also have pervasive effects throughout life (Mousseau & Fox, 1998b).

Maternal effects are increasingly recognized as important influences on the expression and evolution of traits in natural populations, but surprisingly little work has examined how maternal effects may be modified by variation in offspring environments. Environmental heterogeneity is ubiquitous in the wild. For instance, resource availability, parasitism and predation pressure, and exposure to environmental toxins commonly vary over time and space. This environmental variability may substantially change the magnitude or even the direction of the relationship between maternal phenotypes and offspring phenotypes (McAdam & Boutin, 2003; Pakkasmaa et al., 2003; Einum & Fleming, 1999; Carter et al., 2004; Figure 1; Stratton, 1989; Rossiter, 1998). For example, in the frog, *Bombina orientalis*, the amount of food available to developing offspring modified the relationship between egg size (a maternal character) and offspring performance (Parichy & Kaplan, 1992). For many birds, the presence of predators can change the relationship between the frequency of offspring provisioning (a maternal or paternal character) and offspring survival (Skutch, 1949). A higher rate of provisioning increases offspring survival in predator-free environments, while the same behavior can attract predators to nests and result in lower offspring survival in predator-rich environments (Martin et al., 2000; Martin, 1992).
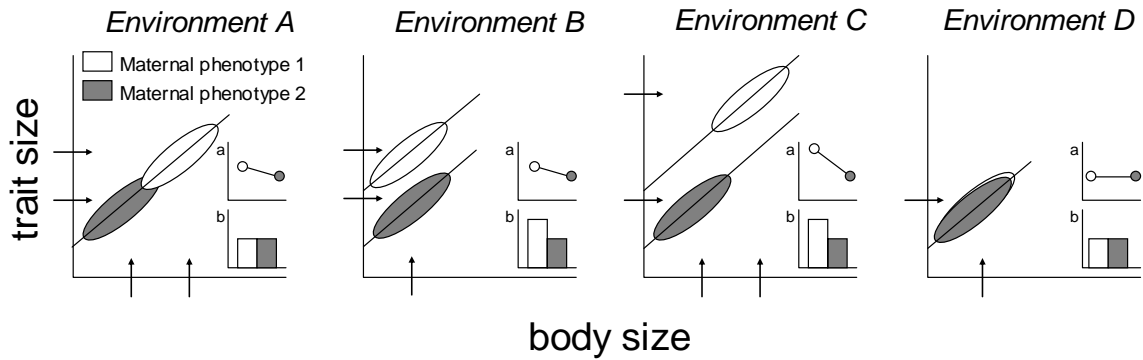
Figure 1. Potential implications of environmental heterogeneity on the expression and magnitude of maternal effects on offspring morphology and allometry. Two different maternal phenotypes (such as two levels of egg size or host plant species choice by ovipositing mothers) may have different effects on offspring depending upon the environmental context (such as resource availability to offspring). In "Environment A" offspring that experience maternal phenotype "1" express larger trait and body sizes, and the allometric intercept between trait size and body size remains the same. In "Environment B" offspring that experience maternal phenotype "1" express relatively bigger traits for their body size (i.e. there is a shift in allometric intercept), though mean body size remains the same. In "Environment C" offspring that experience maternal phenotype "1" have increased trait and body sizes, and also relatively larger trait sizes for their body size. In "Environment D" offspring that experience maternal phenotype "1" do not differ significantly from offspring experiencing maternal phenotype "2". Thus, in this environment, researchers might conclude that there are no maternal effects resulting from variation in the maternal phenotype, even though maternal effects would be present in another environment. Arrows represent trait and body size means. Insets represent (a) mean trait size for offspring experiencing maternal phenotypes "1" or "2" and (b) mean trait size controlling for body size (relative trait size). Environmental effects may also influence allometric slope (not shown).

In this paper we examine the role of seasonal changes in host plants on the magnitude and direction of maternal effects on morphology in the heliconia bug, *Leptoscelis tricolor* (Heteroptera: Coreidae). The selection of host plant species for offspring is a prominent source of maternal effects for many generalist insects, including heliconia bugs (Mayhew, 1997; Thompson, 1988; Awmack & Leather, 2002; C.W. Miller, Chapter 5; Sutherland, 1969a; Jaenike, 1978; Mousseau & Fox, 1998a). Juvenile insects lack wings, and heliconia bugs are restricted to a single host plant and species due to their limited mobility. By the time they can fly away and choose their own host plant, they are adults and their body dimensions are fixed for life. Thus, host plant species choice may have important lifetime consequences for progeny. In addition, these maternal effects may vary temporally with seasonal changes in host plant quality. Natural, phenological changes in the resource availability of host plants have effects on morphology and performance in many insects (Kearsley & Whitham, 1989).

Heliconia bugs in our study area (Gamboa, Panama) commonly use two abundant plant species, *Heliconia platystachys* and *H. mariae*, during the rainy season. These species have different phenologies and as a result they varied in resource availability and quality over our study period (Figure 2). We examined whether the effect of natal host plant species on offspring morphological traits varied over time by examining four separate cohorts of new adults. For each insect, we measured hind femur width (used in male-male competitions), front femur length (used in courtship and male-male competitions), pronotum width (a metric of body size), and head length (a trait with no known use in competition or courtship). In addition, we estimated allometric relationships between morphological traits and overall body size. Such allometric relationships can contain important information regarding the responsiveness of trait growth to body size and individual condition during development (Shingleton et al., 2007; Emlen & Allen, 2003).
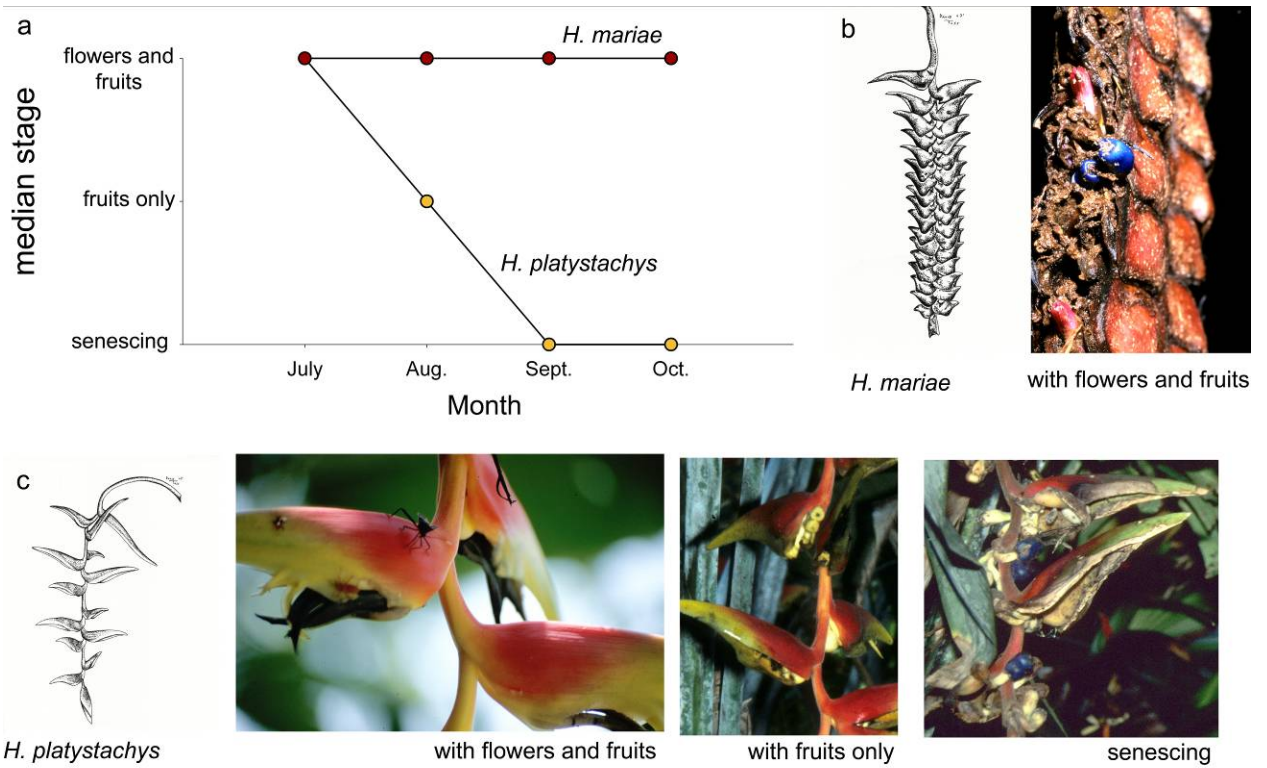
Figure 2. The phenology of two common host plant species, *Heliconia platystachys* and *H. mariae* as measured in 2005 (other years show similar patterns). The median stage of *H. platystachys* inflorescences in the population declines over time, (*a*) and (*c*), while the median stage of *H. mariae* remains constant during this time period, (*a*) and (*b*). Drawings by David Tuss, photographs by Christine W. Miller. Modified from C.W. Miller (Chapter 5).

Methods

<u>Research species</u>

Male heliconia bugs often establish territories on the inflorescences of heliconia plants and will chase away or fight with intruding males. When two males encounter one another, one male commonly begins twitching his front legs up and down. If the other male responds in turn, then both males pivot away from each other and grapple with their hind legs.   One or a series of intense squeezing bouts follow, with each male's hind legs wrapped around the abdomen of his opponent. The interaction terminates when one male disentangles from the other and leaves the area (CW Miller, personal observation). Because sexual selection on body shape has not yet been quantified in this species, secondary-sexual and metric traits are differentiated on the basis of their observed roles in male-male competitions (C.W. Miller, unpublished data).

Females fly from plant to plant, and are often courted by males when they arrive on inflorescences. When one sex approaches the other, males often begin to twitch their front legs up and down, as they do before male-male competitions. The male then begins to tap a female's abdomen with his front legs. A female may respond by moving away, by swinging her abdomen away from the male and hitting him with her front legs, or by allowing the male to copulate. Females will commonly adopt one of the first two behaviors when initially contacted by a male and then, after he has courted her further, allow copulation (CW Miller, personal observations). Females lay single eggs on or very near to heliconia inflorescences, and may continue laying eggs for up to 80 days or longer. Eggs hatch after approximately 10 days, and nymphs molt into adults about a month later.

Heliconia platystachys and *H. mariae* differ markedly in their phenology. *H. platystachys* plants produce inflorescences only once per year, generally during May and June. Over the next few months, these inflorescences progress through distinct phenological stages (Figure 2; CW Miller, Chapter 5), until, by November, no viable inflorescences remain. *H. mariae* plants, in contrast, produce inflorescences in a staggered fashion year-round. Thus, throughout our study period, most *H. mariae* inflorescences had both flowers and fruits available (Figure 2).

Detection and measurements of new adults in the field

During the rainy season of 2005 naturally-growing heliconia inflorescences near Gamboa, Panama were searched for fifth-stage *Leptoscelis tricolor* nymphs. Fine mesh bags were slipped over each inflorescence where a nymph was found. If additional nymphs were present on an inflorescence they were removed and not included in the study. The mesh bag over the inflorescences prevented the insects from leaving the inflorescence when they later emerged as flight-capable adults (up to a week after detection). The first cohort of insects was detected between June 22 and July 1, 2005 (hereafter "July" cohort); the second cohort between July 27 and July 30, 2005 (hereafter "August" cohort), the third between August 24 and September 3, 2005 (hereafter "September" cohort), and the final cohort between September 23 and October 1, 2005 (hereafter "October" cohort).

Insects were monitored until they had emerged as adults and had fully sclerotized cuticles (up to two weeks after detection). We then measured the hind femur width, front femur length, pronotum width, and head length of each adult. Studies incorporating multi-trait comparisons are important for establishing the relative degree of environmental sensitivity of secondary-sexual traits (Cotton et al., 2004b).

We measured the live insects within 0.01mm with digital calipers. Hind femur width was determined by measuring both hind legs at the third distal spine and taking the average of these measurements. Our hind femur width measurement includes both the width of the femur itself and the length of the spine. This is the widest part of the hind femur, and this area frequently comes into contact with the abdomens of rival males during male-male competitions (C.W. Miller, personal observation). Pronotum width was used as a metric of body size because it is a common proxy for body size in insects and easy to measure accurately (~98%). We estimated front femur length by measuring the left front femur from the thorax at the base of the leg to the top of the proximal tip of the tibia. Head length was measured dorsally from the point of connection with the thorax.

Statistical Analyses

We tested for effects of host plant species, sex, month of emergence (cohort) and all possible interactions explaining among-individual variation in insect morphological traits with analysis of variance. We next examined the effects of these factors on the allometric (scaling) relationships between trait size and body size using analysis of covariance with pronotum width as the covariate. First, we performed a separate ANCOVA for hind femur width, front leg length, and head length to test for effects of the explanatory variables on allometric slope, indicated by a significant interaction with pronotum width. Second, we examined effects of explanatory variables on allometric intercept (i.e. trait size when controlling for body size).

We repeated all analyses using both raw and log-transformed data, and all gave qualitatively equivalent results. Thus, we only present the analyses using the raw data.

Results

Trait size and sexual dimorphism

Insects that developed on *H. platystachys* were larger than insects from *H. mariae* for all body size characteristics measured (Table 1; Figure 3). The host plant effect varied over time, however. Most notably, the differences in trait size resulting from alternative host plant species were dramatic early in the season but declined over time so that most body size characteristics did not differ by host plant species by the October cohort (Figure 3).

Table 1. Results of analysis of variance for body size traits

source	d.f.	hind femur width F	front femur length F	pronotum width F	head length F
sex	1	757.81***	8.90**	2.01	0.56
plant species	1	37.66***	32.23***	35.36***	10.94***
cohort	3	4.56**	5.61**	4.86**	10.68***
sex x plant	1	17.38***	3.29	0.69	4.73*
sex x cohort	3	1.50	0.79	0.54	0.18
plant x cohort	3	4.02**	3.46**	5.18**	1.26
sex x plant x cohort	3	2.61	0.38	0.38	2.43

Error df = 247 - 250

*$P < 0.05$, ** $P < 0.02$, *** $P < 0.001$.*
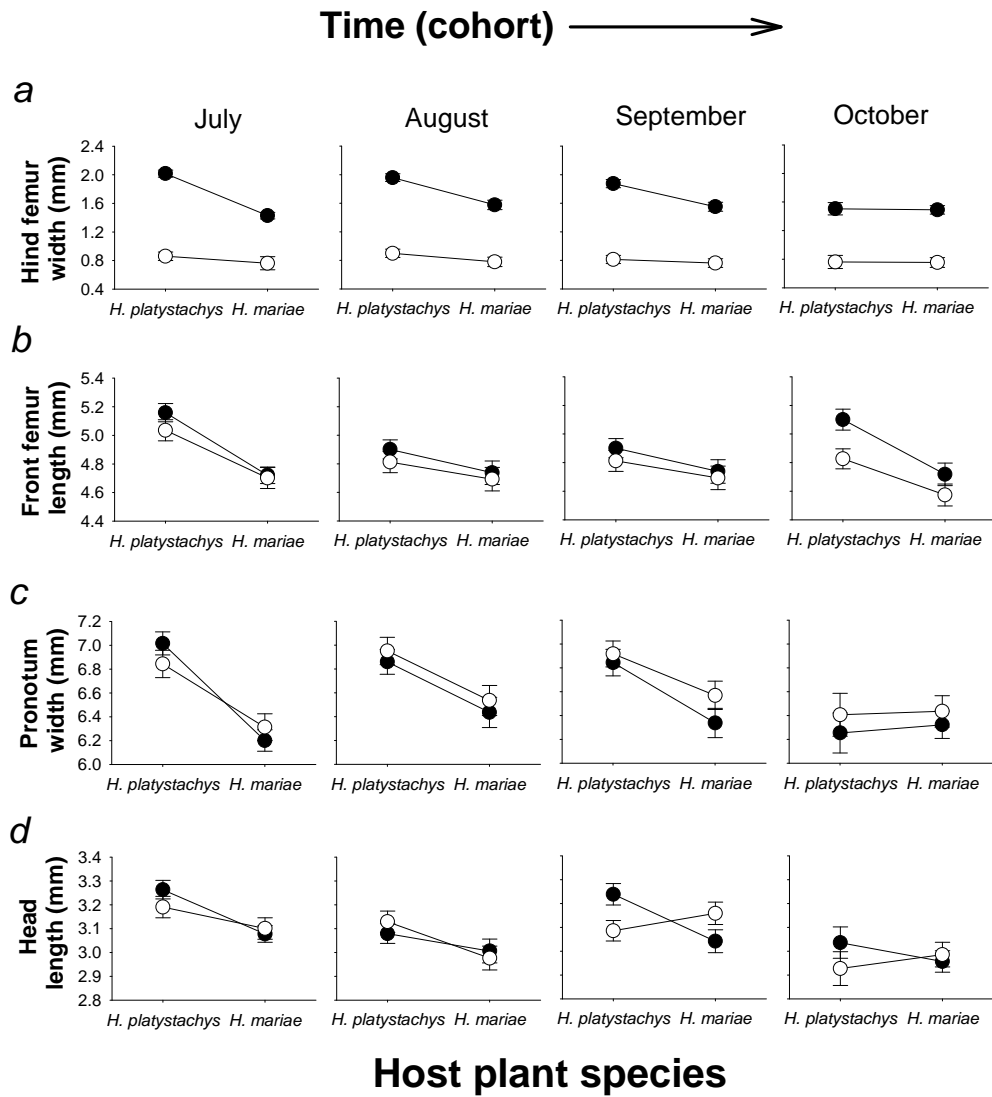
Figure 3. Morphological traits (a-d) for males (solid circles) and females (hollow circles) for each of the separate cohorts of insects (least-squares means $\pm$ standard errors).

Sexual dimorphism was found in hind femur width, front leg length, and head length. Relative to females, males had both absolutely and relatively larger hind femurs equipped with prominent spines. Males also had front femurs that were relatively longer than those of females. Relative head length was sexually dimorphic, but the nature of the dimorphism varied (see below).

The magnitude and even the direction of sexual dimorphism were affected significantly by nymphal host plant, i.e. by maternal effects. Interestingly, each sexually dimorphic characteristic in these insects exhibited a unique response to environmental variation (Table 1; Figure 3). The degree of sexual dimorphism in hind femur width was greater on *H. platystachys* than on *H. mariae* (Figure 3). In contrast, front femur length did not vary in the degree of sexual dimorphism either between plant species or across cohorts (Figure 3b). For head length, the direction of sexual dimorphism reversed between host plant species (Table 1; Figure 3d). Males that developed on *H. platystachys* had longer heads than females, whereas for bugs that developed on *H. mariae,* females had longer heads than males. This pattern was especially pronounced in the September cohort.

Trait allometry (scaling relationships)

The allometric slope between femur width and body size (pronotum width) differed by sex (Table 2). Therefore, we tested for allometric intercept differences (i.e. trait size relative to body size) of hind femur width separately for males and females. Natal host plant species had significant effects on the relative size of hind legs for both males and females, though the effects on males were more pronounced (Table 3; Figure 4). Separate analyses by cohort revealed that males that emerged in July and August develop larger legs relative to body size if they are raised on *H. platystachys* (ANCOVA: July, $F_{1,49} = 10.41$, $p = .002$; August: $F_{1,42} = 5.43$, $p = .026$; Figure 4a). In the September and October cohorts, males raised on the two species of plants did not differ in relative hind femur size (ANCOVA: September, $F_{1,30} = 0.37$, $p > 0.10$; October: $F_{1,22} = 0.74$, $p > .10$; Figure 4a). Surprisingly, female natal host plant species was also important for relative leg sizes. Females emerging from *H. platystachys* in August had larger legs relative to body size than females from *H. mariae* (ANCOVA: $F_{1,28} = 28.37$, $p < 0.001$;

Figure 4b). In no other cohort did natal host plant species have a detectable effect on female relative leg size (ANCOVA: July $F_{1,32}= 0.53$, $p > 0.10$; September, $F_{1,32}= 0.01$, $p > 0.10$; October: $F_{1,17}= 0.23$, $p > .10$; Figure 4b).

Table 2. Results of analysis of covariance for effects on allometric slope with body size (pronotum width) as a covariate

source	d.f.	hind femur width F	front femur length F	head length F
body size (pw)	1	373.16***	120.77***	41.16***
sex	1	86.04***	0.04	0.02
plant species	1	0.07	0.30	0.61
cohort	3	1.15	0.38	1.23
body size x sex	1	178.87***	0.28	0.01
body size x plant	1	0.01	0.10	0.70
body size x cohort	3	0.90	0.39	0.87
sex x plant	1	0.73	0.06	0.01
sex x cohort	3	0.49	0.91	1.91
plant x cohort	3	3.21*	1.69	0.93
sex x plant x cohort	3	1.01	1.36	2.23
body size x plant x cohort	3	2.88*	1.61	0.91
body size x plant x sex	1	1.04	0.02	0.03
body size x cohort x sex	3	0.51	0.81	1.84

Error df = 231 - 241
*P < 0.05, ** P < 0.02, *** P < 0.001.*

Table 3. Results of analysis of covariance for the allometric intercept of hind femur width with body size (pronotum width) as a covariate. The analyses were performed separately for males and females.

source	Males d.f.	Males F	Females d.f.	Females F
body size (pw)	1	497.48***	1	83.52***
plant species	1	11.30***	1	5.93*
cohort	3	0.93	3	8.67***
plant x cohort	3	1.06	3	2.39

Error df: Males = 135; Females =112
*P < 0.05, ** P < 0.02, *** P < 0.001.*
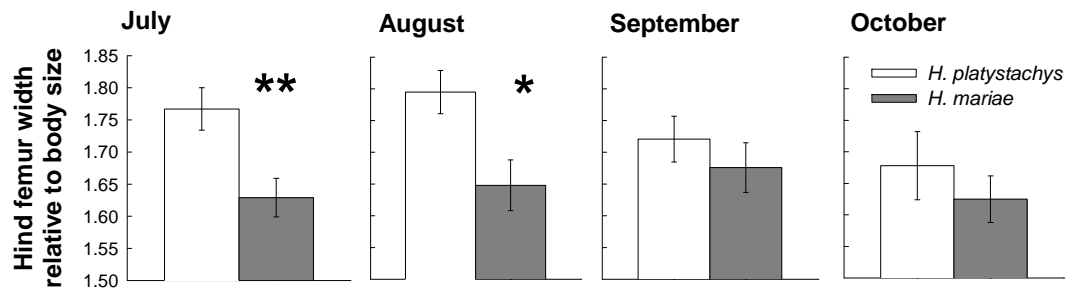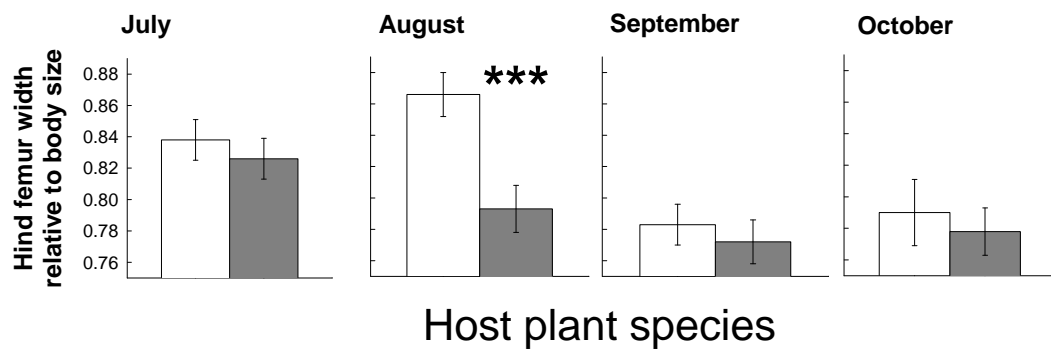
Figure 4. Relative hind femur width for males (a) and females (b), over four cohorts (least-squares means ± standard errors). Asterisks denote significant differences when each cohort was analyzed separately (** P < 0.05, ** P < 0.02, *** P < 0.001*).

The allometric slopes of head length and front leg length were not significantly affected by plant species, sex, or cohort. Therefore, we proceeded to look for effects of the explanatory variables on allometric intercept (trait size relative to body size). Front leg length relative to body size was significantly impacted by host plant species (Table 4; Table 5). Those insects raised on *H. platystachys* had relatively longer front femurs for their body size, however, the investment in front femurs was more pronounced for males (Table 5). Relative head length was not significantly affected by host plant species (Table 4, Table 6).

Table 4. Results of analysis of covariance for allometric intercept of head length and front femur length in males and females

source	d.f.	Front femur length F	Head length F
body size (pw)	1	153.85***	57.06***
sex	1	24.24***	2.33
plant species	1	5.94**	0.63
cohort	3	6.34***	10.09***
sex x plant	1	2.69	4.00*
sex x cohort	3	2.63	0.29
plant x cohort	3	1.69	0.25
sex x plant x cohort	3	1.14	2.79*

Error df = 246

*P < 0.05, ** P < 0.02, *** P < 0.001.*

Table 5. Means ± SE for front femur length relative to body size

cohort	Males		Females	
	H. platystachys	*H. mariae*	*H. platystachys*	*H. mariae*
July	4.99 ± .05	4.88 ± .05	4.94 ± .06	4.81 ± .06
August	4.80 ± .05	4.81 ± .07	4.67 ± .06	4.72 ± .07
September	5.01 ± .06	4.82 ± .06	4.70 ± .06	4.59 ± .06
October	4.98 ± .09	4.74 ± .06	4.64 ± .09	4.71 ± .07

Table 6. Means ± SE for head length relative to body size

cohort	Males		Females	
	H. platystachys	*H. mariae*	*H. platystachys*	*H. mariae*
July	3.19 ± .04	3.15 ± .03	3.15 ± .04	3.15 ± .04
August	3.04 ± .04	3.04 ± .05	3.07 ± .04	2.99 ± .05
September	3.20 ± .04	3.09 ± .04	3.03 ± .04	3.17 ± .04
October	3.10 ± .06	3.01 ± .04	2.96 ± .06	3.02 ± .05

DISCUSSION

Maternal effects from choice of host plant species

      Maternal choice of *Heliconia platystachys* for oviposition resulted in offspring that were larger (Figure 3; Table 1) and had relatively larger hind femurs and front femurs for their body size than did males and females from *H. mariae* during this time period (Figure 4; Tables 3-6). These maternal effects may have important implications for male offspring reproductive success because males use these traits in intra-sexual contests and in courtship. The host plant species a mother bug chooses may also affect the reproductive success of her female offspring, because female invertebrate size and fecundity is often strongly correlated (Roff, 1992). Indeed, females from *H. platystachys* during this time period had higher initial fecundity than did females from *H. mariae* (C.W. Miller, Chapter 5).

The changing consequences of maternal host plant species choice

      The influence of natal host plant species on offspring phenotypes changed over the seasonal phenology of the plants (Figures 3, 4; Tables 1 – 5). Early cohorts of offspring developing on *H. platystachys* were generally larger, more sexually dimorphic, and had larger femurs relative to body size than insects that developed on *H. mariae*. However, the October cohort of offspring from *H. platystachys* and *H. mariae* were virtually indistinguishable for most characteristics. Hence there was a strong interaction between a maternal phenotype (host plant choice) and other environmental factors in shaping offspring morphological characteristics.

      We have also examined reproductive behaviors in these cohorts of insects, and found that host plant species choice by mothers influenced the probability of mating, female fecundity, and performance of copulatory courtship behaviors by males (C.W. Miller, Chapter 5). Maternal effects on offspring behaviors were also significantly modified by seasonal changes, as we found here. These studies, along with quantitative genetics data demonstrating low heritabilities of morphological traits in this species (Appendix 1), emphasize the importance of maternal phenotypes and environmental context in determining offspring phenotypes in this species.

The meaning behind complex patterns of trait expression

This study reveals a rich natural setting where maternal host plant species choice combined with host plant phenology result in dynamic patterns of offspring trait expression over time. Because the patterns examined here are those naturally occurring in a wild population of insects, they are relevant to a wide breadth of natural phenomena. Variation in morphology may influence the outcome of natural and sexual selection by directly affecting the outcomes of male-male competitions and female choice of mates. In addition, this variation may reflect the overall physical condition of both males and females (see also C.W. Miller, Chapter 3), with likely implications for individual fitness.

Studies have increasingly suggested that secondary-sexual traits, like the ones measured here, are highly sensitive to environmental factors, including the phenotypes of mothers (Qvarnström & Price, 2001; Griffith et al., 1999; e.g. Emlen, 1997; Moczek & Emlen, 1999). Therefore, the expression of ornaments (used to attract mates) or weapons (used in intrasexual contexts) may more accurately reflect maternal phenotypes and the environments experienced than direct genetic factors. However, most theoretical work on the evolution of secondary-sexual traits has focused primarily on the direct genetic factors underlying the expression of these traits. This work likely ignores the environmental variance that contributes to these traits because *random* environmental variation is not expected to alter the direction of evolutionary change. Indeed, in laboratory studies, environmental variation in these traits may appear random or be primarily under the control of researchers. However, in natural situations, offspring environments (and, thus, trait expression) may commonly be shaped by mothers.

What do maternal effects mean for evolutionary change?   When offspring traits are shaped by mothers, as they are here, the evolutionary response to selection may be slower, faster, or even in the opposite direction to that expected (Kirkpatrick & Lande, 1989; Lande & Kirkpatrick, 1990). Further work is needed to explore the potential evolutionary dynamics that may occur natural environmental variation affects the expression of maternal effects in offspring.

Why do mothers choose H. *mariae* when *H. platystachys* appears to be superior for offspring ?

When maternal phenotypes influence the phenotypes of offspring, natural selection may improve the ability of mothers to choose the best inflorescences available at any one time. However, theory predicts that mothers will often have conflicting fitness demands, and should enhance their own fitness even at a cost to individual offspring (Mayhew, 1997; Mayhew, 2001; Scheirs et al., 2000; Nylin & Janz, 1996; Godfray, 1995). This may explain why females continue to use *H. mariae* early in the season even when ample *H. platystachys* inflorescences are available (C.W. Miller, personal observation). Patches of these two plant species only rarely grow in close proximity, thus considerable travel is required to reach *H. platystachys* as it becomes available as a resource. The expenditure of time and energy, and the risk of predation by birds and spiders may select against females that make the journey. These fitness trade-offs may help maintain genetic variation in maternal phenotypes and thus sustain the considerable phenotypic variation in offspring morphological trait expression.

Conclusions

Maternal effects are ubiquitous and have important implications for the expression of traits, ecological dynamics, and evolutionary change. Therefore predicting where and when they should occur will be an asset to many fields of biology. However, few studies have yet explored how natural environmental heterogeneity may enhance, buffer, or even reverse the direction of maternal effects. Here, we have found that seasonal changes modify the relationship between a maternal phenotype, host plant species choice, and offspring morphology. More work is needed to examine patterns of maternal effects expression in multiple natural contexts and to extrapolate results to larger-scale processes.

CHAPTER 4

CONDITION-DEPENDENT TRAIT EXPRESSION IN NATURAL ENVIRONMENTS

Abstract

The expression of secondary-sexual traits is often extremely variable in natural populations and may result in large differences in individual fitness. Such variation is thought to reflect differences in the condition of individuals, and individuals in good condition have commonly been assumed to be of good genetic quality. However, in natural situations, individual condition and secondary-sexual trait expression are often largely determined by environmental factors. I investigated the effects of different natal host plant species on absolute and relative expression of several adult morphological traits in the heliconia bug, *Leptoscelis tricolor*, in natural settings. The host plant species where insects developed affected the absolute size of adult traits. The degree of sexual dimorphism also differed between the two host plant species. Furthermore, males raised on the two host plant species varied in hind femur size relative to body size. However, the expression of other traits relative to body size was not affected. Males may therefore invest particularly heavily in hind femur width, a trait used by males in intrasexual contests, when higher quality resources are present. These patterns suggest that male hind femur width is condition dependent and a sensitive indicator of male phenotypic quality. Strong environmental influences on trait expression may have important consequences for male-male competitions, reproductive success, and ultimately, sexual selection.

Introduction

Secondary-sexual traits, including horns, pheromones, and colorful plumage, are the most variable traits in natural populations (e.g. Andersson, 1994; Darwin, 1874; Thornhill & Alcock, 1983). The phenotypic variation in these traits can be puzzling, because they are often critical to fitness and directional selection on these traits should be strong, thereby reducing among-individual variation in expression (Andersson, 1994). Why don't all males exhibit large, elaborate secondary-sexual traits?

The recognition that the expression of secondary-sexual traits is often dependent upon male phenotypic condition helps resolve this dilemma. Individuals in good condition are predicted to express more elaborate secondary-sexual traits than individuals in poor condition (Lorch et al., 2003; Tomkins et al., 2004; Cotton et al., 2004b; see also Rowe & Houle, 1996; Bonduriansky & Rowe, 2005). These traits should therefore accurately reflect condition, and may be signals for conspecifics in mate choice decisions and in competitive contexts (Andersson, 1982; Andersson, 1994; Johnstone, 1995; Kodric-Brown & Brown, 1984; Nur & Hasson, 1984; Zeh & Zeh, 1988).

The notable variation in secondary-sexual trait expression may therefore be largely due to differences in individual condition. But, what is responsible for this variation in condition? Genetic contributions to condition have received much attention (Rowe & Houle, 1996; Tomkins et al., 2004). However, environmental factors, especially in nature, are often the primary source of among-individual variation in condition (Price & Schluter, 1991). Thus, males expressing elaborate secondary-sexual traits and achieving high reproductive success may be those that experienced good environments. Environmental influences on secondary-sexual trait expression may have important, and relatively unexplored, implications for the evolution of these traits (for one example, see Miller & Moore, 2007).

Interestingly, while researchers often discuss the likely importance of genes in determining individual condition, most empirical studies of condition dependence use laboratory manipulations of nutrition or environmental stress to establish condition. These studies of secondary-sexual trait expression sometimes find perplexing patterns of condition dependence. Because the vast majority of studies on condition dependence

have been conducted under artificial laboratory conditions, it is difficult to determine whether results are representative of natural patterns of trait expression or are simply artifacts of unrealistic environments (Cotton et al., 2004b). Therefore, examining condition dependence of traits in natural populations is a necessary complement to existing laboratory studies. By using different *natural* contexts to examine the condition dependence of secondary-sexual traits, the observed patterns of trait expression are more assuredly relevant to the species' ecology and its evolution.

Here I take a novel approach to investigating condition dependence by using natural environmental variation to examine levels of condition-dependent trait expression. Many individuals in natural populations experience discretely different environments while growing and developing. For example, generalist insects oviposit on one of a few potential host plant species, and juveniles are often restricted to the plant where they hatch because of their limited mobility. Thus, these insects provide excellent opportunities to compare natural patterns of trait expression across developmental environments. Growing up on different host plant species often results in measurable variation among insects in fitness-related traits such as survival, developmental rate, adult size, fecundity, and fertilization success (Thompson, 1988; Awmack & Leather, 2002; C.W. Miller, Chapter 5; Jaenike, 1978; Rausher, 1980; Rausher, 1981; Sutherland, 1969b; Mayhew, 1997; Janz et al., 1994; Scribner & Slansky Jr. , 1981; Wedell, 1996). While much insightful work has been conducted in such natural systems, few studies have used the distinct environments provided by different host plant species to examine patterns of condition-dependent trait expression.

I investigated the influence of two naturally-growing host plant species on trait expression in the heliconia bug, *Leptoscelis tricolor* (Heteroptera: Coreidae). Heliconia bugs feed, mate, and lay eggs on the inflorescences of heliconia plants in Panama and Costa Rica. Females fly from inflorescence to inflorescence, and may lay eggs on several heliconia species over their lifetimes. The plant where a mother lays eggs largely determines the natal environment for offspring. Juvenile insects lack fully-developed wings and do not fly. Walking is unlikely to take a young heliconia bug to a different species of plant because of the often appreciable distances separating the mono-specific

stands of heliconia. Thus, the natal host plant species of an individual can be determined by noting the plant on which a bug is found before it has reached maturity.

Males commonly establish territories on heliconia inflorescences and will chase away or fight with intruding males. When two males encounter one another, one male commonly begins twitching his front legs up and down, displaying his front femurs to the other male (Figure 1). If the encounter escalates, the males turn around and grapple with their hind legs. These competitions often terminate after one or a series of intense squeezing bouts with each male's hind legs wrapped around the abdomen of the other male. Male femurs are adorned with spines that contact the opponent's abdomen during competitions (Figure 2, C.W. Miller, personal observation). Front femur length and hind femur width were thus considered secondary-sexual traits on the basis of their observed use in sexual contexts.

Figure 1. Two males initiating a competition and displaying their front legs.
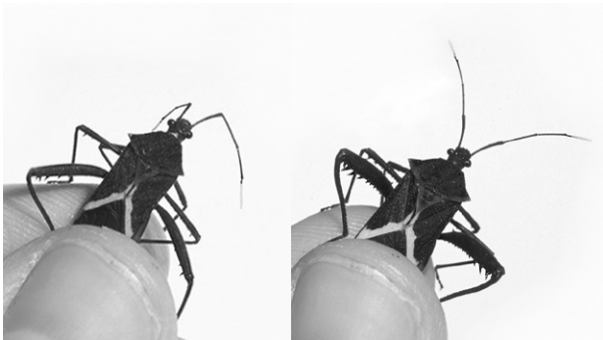
Figure 2. Body size differences. The male on the left emerged from *H. mariae*, and the male on the right emerged on *H. platystachys*. Note the visible differences in hind femur width.

Previous work has suggested that secondary-sexual traits in heliconia bugs in this population have low heritabilities (Appendix 1) and extreme sensitivity to host plant environments (C.W. Miller, unpublished data). Thus, I had an *a priori* reason to suspect that the different host plant species could strongly influence individual condition. I measured several morphological traits on individual bugs raised on two species of heliconia plants to determine relative levels of condition dependence of secondary-sexual and metric traits in this species.

Methods

<u>Assessing levels of condition dependence</u>

Most traits demonstrate some degree of allometric scaling with body size. Thus, to examine effects of condition on trait expression, the effect of body size should be removed by including it as a covariate in general linear models (Darlington & Smulders, 2001; Garcia-Bertou, 2001; Cotton et al., 2004b). Patterns of condition dependence may take a variety of forms (Figure 3), with secondary-sexual traits predicted to exhibit a heightened sensitivity to condition than normal, metric traits. This heightened condition dependence is generally expected to be revealed by a shift in the allometric relationship between body and trait sizes for animals of different levels of condition (Figure 3c). However, condition dependence may also be revealed by changes in allometric slope (Figure 3d). I test these alternatives for secondary-sexual and metric traits.
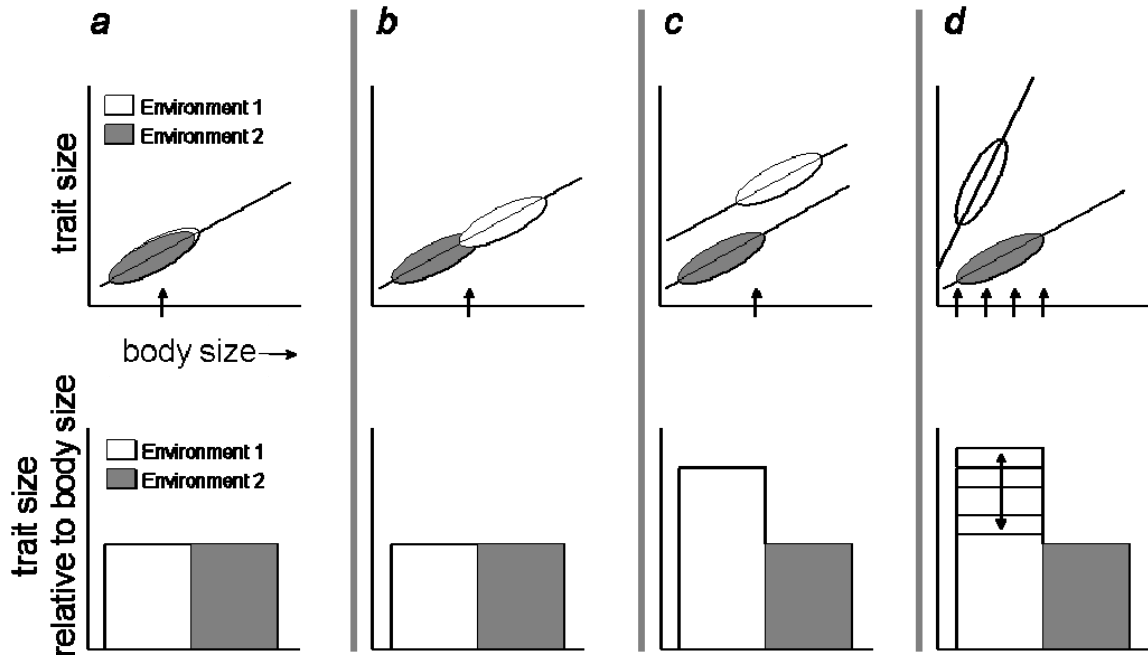
Figure 3. Hypothetical effects of juvenile environmental differences on adult morphological traits. The top row show allometric scaling relationships between body size and a hypothetical trait. The bottom row reflects this same data, but plots trait size relative to body size. In (a) neither body size nor trait size responds to environmental variation. In this case, the environmental treatments either did not affect the condition of the animals or the trait is not condition dependent. In (b) individuals raised in Environment 1 are larger in both body and trait size, but the relationship between body size and trait size remains the same. This pattern of trait expression suggests moderate condition dependence, expected in normal, "metric" traits, where trait size reflects body size. In (c) individuals raised in Environment 1 are larger in both body and trait size, and there is a shift in the allometric scaling relationship so that for any given body size, individuals raised in Environment 1 have a larger trait size than individuals raised in Environment 2. This pattern is commonly used to infer heightened condition dependence, and is the expected result for secondary-sexual ornaments or weapons. In (d) the allometric slope of trait size relative to body size varies for animals raised in different environments. Here, trait size relative to body size is different for the two groups, and the difference also varies with body size. This pattern of trait expression may allow large, high condition individuals to express exceptionally large traits. This pattern also reflects condition-dependent trait expression, however further work on precise interpretations is needed.

Detection and measurements of new adults in the field

From June 22nd to July 1st, 2005 my assistants and I searched naturally-growing heliconia inflorescences near Gamboa, Panama for fifth-stage *Leptoscelis tricolor* nymphs. When we detected a nymph, we used a fine mesh bag to enclose the individual and its host inflorescence. We removed any additional nymphs (generally 0 to 3) so that only one individual was enclosed in each bag. When the bagged nymphs molted into flight-capable adults (up to seven days later), the mesh bags prevented them from leaving the host plant.

My assistants and I returned after insect had emerged to collect and measure the new adults. I measured live insects within 0.01mm with 6" digital calipers for body size (pronotum width) and three additional morphological traits, including one with a direct role in male-male competitions (hind femur width), one used in signaling prior to competitions and in courting females (front femur length; Figure 2), and one with no known role in competitions or courtship (head length).

Pronotum width was used as a metric of body size because it is a common proxy for body size in insects and easy to measure with high accuracy (~98%). Hind femur width was determined by taking the mean width of both hind legs at the third distal spine. This measurement includes both the width of the femur itself and the length of the spine. This is the widest part of the hind femur, and this region of the leg frequently comes into contact with the abdomens of other males in male-male competitions (C.W. Miller, personal observation). I estimated front femur length by measuring the left front femur from the thorax at the base of the leg to the top of the proximal tip of the tibia. Head length was measured dorsally from the point of connection with the thorax.

Statistical Analyses

I tested the significance of host plant species in explaining variation in the expression of insect morphological traits with analysis of variance using host plant species, sex, and their interactions as explanatory variables. To examine host plant effects on relative trait size (i.e. body shape characteristics) I used analysis of covariance with pronotum width as the covariate. I tested for host plant species and sex effects on allometric slope and

allometric intercept by performing ANCOVAs with pronotum width as the covariate. Analyses were performed separately for hind femur width, front femur length, and head length. I first tested for effects on allometric slope, indicated by a significant interaction with pronotum width. I next examined effects of explanatory variables on allometric intercept (i.e. trait size when controlling for body size) by performing ANCOVAs with all (nonsignificant) interactions with pronotum width removed from the model.

Heightened condition dependence was evaluated as a significant change in relative trait size across host plants (Figure 3). I conducted all analyses using raw and log-transformed data, and all gave qualitatively equivalent results. Thus, I only present the analyses with the raw data. With these same statistical techniques, I was also able to evaluate whether the degree of sexual dimorphism in males and females varied by host plant species (detected as a significant host plant x sex interaction).

Results

Heliconia host plant species had significant effects on adult body size (Table 1; Table 2; Figure 4). Males and females emerging from *H. platystachys* were larger in all absolute trait sizes. In addition, insects from *H. platystachys* exhibited greater sexual dimorphism in hind femur width than those from *H. mariae* (Table 1; Figure 4).

Table 1. Results of analyses of variance for trait sizes in males and females

source	Pronotum width d.f.	F	Hind femur width d.f.	F	Front leg length d.f.	F	Head length d.f.	F
host plant	1	54.35***	1	54.46***	1	36.44***	1	14.04***
sex	1	0.11	1	403.52***	1	1.24	1	0.49
host plant x sex	1	2.43	1	33.31***	1	0.67	1	1.70

*$P < 0.05$, ** $P < 0.02$, *** $P < 0.001$.*

Table 2. Means $\pm$ SE for morphological traits in males and females. See also Figure 5.

trait	Males H. platystachys	H. mariae	Females H. platystachys	H. mariae
pronotum width	7.02 $\pm$ .09	6.20 $\pm$ .08	6.84 $\pm$ .10	6.31 $\pm$ .10
front femur length	5.16 $\pm$ .06	4.72 $\pm$ .05	5.04 $\pm$ .07	4.70 $\pm$ .07
head length	3.27 $\pm$ .04	3.08 $\pm$ .03	3.19 $\pm$ .04	3.10 $\pm$ .04
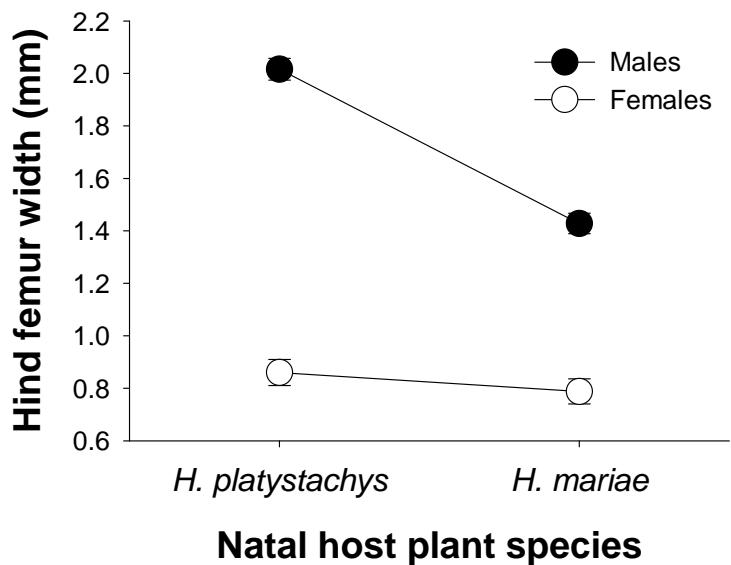
Figure 4. Hind femur width for males and females emerging from *H. platystachys* and *H. mariae* with least-squares means $\pm$ standard errors. Insects emerging from *H. mariae* are significantly less sexually dimorphic ($F_{1,83}$ = 33.31, $P < 0.001$).

I next performed analyses of covariance to test for heterogeneity of allometric slopes. Only hind femur width exhibited sex differences on allometric slope (sex x body size interaction; $F_{1,81} = 34.147$, $P < 0.001$). Thus, further analyses of relative hind femur width were performed separately for males and females. Plant species did not affect the allometric slope of any traits measured.

I tested for effects of plant species on the allometric intercept of morphological trait size relative to body size. This analysis allowed for the effect of body size on morphological trait size to be removed, so effects of condition on trait expression could be more clearly examined. Only the allometric intercept of male hind femur width was significantly affected by host plant species; males from *H. platystachys* had relatively wider femurs for their body size than males from *H. mariae* (male hind femur width: $F_{1,49} = 10.41$, $P = 0.002$; female hind femur width: $F_{1,32} = 0.53$, $P = 0.471$; front leg length: $F_{1,81} = 2.53$, $P = 0.116$, head length $F_{1,81} = 1.68$, $P = 0.199$; Figure 5). This pattern suggests that male hind femur width exhibits heightened condition dependence (see Figure 3).

a) Males

Hind femur width

Front leg length

Head length

Mean trait size relative to body size

H. platystachys
H. mariae

Natal host plant species

** **

Natal host plant species

Natal host plant species

b) Females

Hind femur width

Front leg length

Head length

Mean trait size relative to body size

H. platystachys
H. mariae

Natal host plant species

Natal host plant species
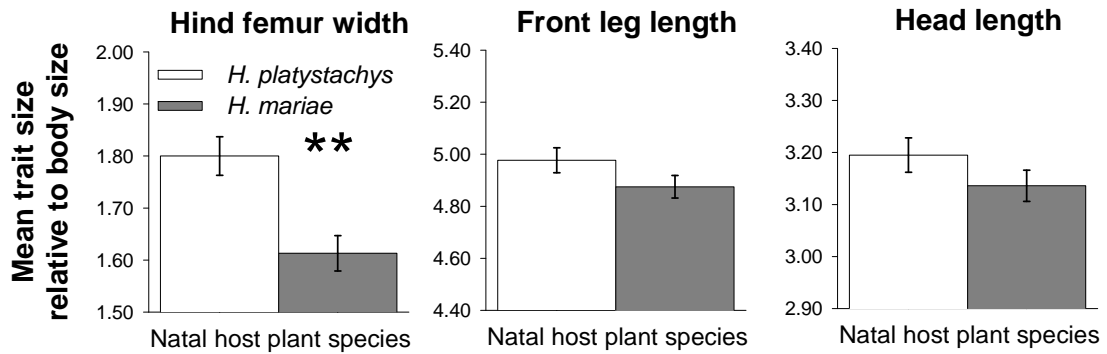
Natal host plant species
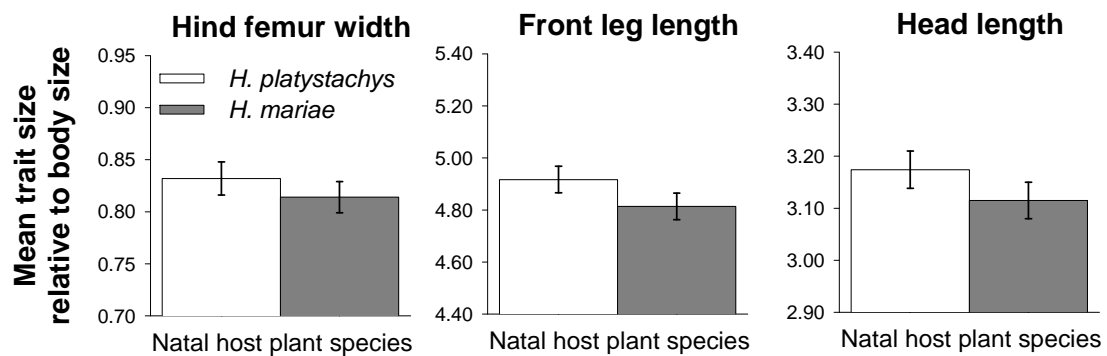
Figure 5. Trait size relative to body size for males (a) and females (b) showing least-squares means $\pm$ standard errors. Only male hind femur width shows indication of heightened condition dependence in response to natural variation in natal host plant species.

Heliconia bugs are highly sexually dimorphic in both hind femur width and the allometric slope of hind femur width relative to body size (hind femur width: Table 1, Figure 4; allometric slope: see above). While the degree of sexual dimorphism in absolute hind femur width was affected by host plant species (see above; Table 1; Figure 4), I found no evidence that the allometric slope of hind femur width relative to body size or other morphological characteristics varied in sexual dimorphism across plant species (allometric slope of hind femur width: $F_{1,79} = 0.02$, $P = 0.901$; front leg length: Table 1; front leg length relative to body size: $F_{1,81} = 1.42$, $P = 0.236$; head length: Table 1; head length relative to body size: $F_{1,81} = 0.36$, $P = 0.551$).

Discussion

This study demonstrates that the expression of male hind femurs, a secondary-sexual trait used by males in intrasexual competitions, was highly sensitive to developmental environments. Males reared on *Heliconia platystachys* not only expressed larger hind femurs than males from *H. mariae*, but the size of these femurs relative to body size was also greater (Figure 5). No other traits measured in males or females were significantly different across environments in expression relative to body size. These results suggest that male hind femurs exhibit greater condition dependence than do other traits (Table 1, Figure 3). Thus, femur size in male heliconia bugs may serve as a sensitive indicator of condition.

The lack of heightened condition dependence in male front femur length is surprising. Males often twitch their front legs up and down when approaching other males (for competitions) and females (for copulations), and in the process display the length of their front femurs. Such lack of condition dependence in traits used in sexual contexts is not without precedence (e.g. Bonduriansky, 2007; David et al., 2000; David et al., 1998; Møller & Petrie, 2002; Cotton et al., 2004a; Cotton et al., 2004b). However, in larger and longer-term analysis of trait expression in this species, I did find evidence of condition dependence in front leg size (C.W. Miller, Chapter 2). I also found that front femur length exhibited sexual dimorphism, which was not found in this study. The

conflicting results suggest that front legs may be more sensitive to certain study environments than others. Whether environmental variation can influence the relationship between condition and the expression of condition dependence is virtually unknown. However, in different contexts, the advantage of expressing large, elaborate morphological traits may vary. Thus, allocation decisions may also vary by context, with high condition individuals expressing large traits in some situations and exceptionally large traits in other situations.

Condition dependence and sexual dimorphism

Theory suggests that traits targeted by sexual selection should evolve both heightened condition dependence and sexual dimorphism (see Bonduriansky, 2007). Sexual dimorphism is thought to evolve when the sexes experience divergent selection pressures, with one sex (generally the male) displaced from the viability-selected optimum by sexual selection. These same secondary-sexual traits are also expected to evolve heightened condition dependence, with individual males expressing the traits to the greatest extent possible for their physical condition. This coevolution of sexual dimorphism and condition dependence should result in distinct patterns of trait expression in different environments. Higher levels of sexual dimorphism are expected for organisms experiencing a high-quality environment than those experiencing a lower-quality environment. However, empirical work has only begun to directly examine whether environmental factors modulate both the degree of sexual dimorphism and the condition dependent expression of secondary-sexual traits (Weladji et al., 2005; Karan et al., 2000; Bonduriansky, 2007; Post et al., 1999; David et al., 1994).

Here, I have found that males and females raised on *H. mariae* were less sexually dimorphic than those raised on *H. platystachys*. The difference in the level of sexual dimorphism was only significant for hind femur width, the same characteristic that exhibited heightened condition dependence. Therefore, the expression of this secondary-sexual trait and the level of sexual dimorphism of males emerging from *H. platystachys* are greater than for males emerging from *H. mariae* during this time period (Figure 3).

Interestingly, these patterns of trait expression may have dynamic, population-level consequences. *H. platystachys* inflorescences are only available for a limited time each

year, while *H. mariae* is available year-round (C.W. Miller, Chapter 5), thus the availability of large males with extremely sexually-dimorphic secondary-sexual traits likely fluctuates over time. The implications for success in intra-sexual contests, mate choice, and resulting reproductive success remain to be investigated.

Conclusions

Increasing evidence suggests that secondary-sexual characteristics are especially sensitive to environmental variation. However, patterns of trait expression have at times been puzzling: sometimes environmental factors influence the allometric slope of the relationship between trait size and body size, sometimes they influence the allometric intercept (i.e. trait size relative to body size, an indication of heightened condition dependence; Figure 1), and sometimes secondary-sexual traits appear to not be conditionally expressed (Cotton et al., 2004a; e.g. Bonduriansky, 2007; David et al., 2000; David et al., 1998; Møller & Petrie, 2002; Cotton et al., 2004b; Cotton et al., 2004c). In addition, non-sexual traits may exhibit heightened condition dependence, contrary to existing predictions (e.g. Bonduriansky & Rowe, 2005). Therefore, further empirical and theoretical work is necessary to predict when and where to expect condition dependence and to better understand the biological significance of these extreme patterns of trait plasticity and condition sensitivity.   The reason for the presence of such diverse patterns of condition dependence is only beginning to be explored (Bonduriansky, 2007; Bonduriansky & Rowe, 2005). These challenges are part of the larger problem of integrating developmental plasticity with evolutionary theory (West-Eberhard, 2003; West-Eberhard, 1986; West-Eberhard, 1989)

I argue that the use of natural environments in examining condition dependence will be a worthwhile tool. By measuring trait expression in natural environments, realistic and relevant patterns will emerge that will help direct future research. In addition, an appreciation of natural contexts is essential for understanding natural patterns of trait expression and predicting the evolution of secondary-sexual traits, condition dependence, and sexual dimorphism.

CHAPTER 5

DYNAMIC MATERNAL EFFECTS ON OFFSPRING REPRODUCTIVE TRAITS

Abstract

Maternal decisions commonly influence offspring phenotypes, and such maternal effects can even have permanent influences on the fitness-related traits of offspring. Importantly, the relative strength and direction of maternal effects can change over time as external environmental factors change, however this phenomenon is virtually unexplored. I examined the changing effects of maternal host plant choice on the mating behaviors and fecundity of four sequential cohorts of adult offspring in the heliconia bug, *Leptoscelis tricolor* (Hemiptera: Coreidae). Female heliconia bugs choose among multiple host plant species for oviposition, and in doing so, they largely determine the resources that offspring will experience as they grow and develop. I found that the probability of mating, expression of copulatory courtship behaviors, and fecundity all differed according to the host plant where mothers laid eggs. Moreover, the relative effects of natal host plant species on daughter reproductive traits shifted and even reversed over time, and these changes were correlated with seasonal changes in plant resources. These results demonstrate that maternal effects may be a large and dynamic source of natural variation in offspring mating behavior and fecundity.

Introduction

The decisions of mothers commonly influence offspring phenotypes and can have large fitness consequences (Marshall & Uller, 2007; Mousseau & Fox, 1998a; Mousseau & Fox, 1998b). The choice of habitat for offspring is one such maternal decision that affects organisms across a variety of taxa (e.g. Mayhew, 1997; Caut et al., 2006; Kolbe & Janzen, 2002; Brittingham & Temple, 1996). In herbivorous insects, habitat selection for offspring often means choosing among different host plant species for offspring (Mayhew, 1997; Thompson, 1988; Awmack & Leather, 2002; Sutherland, 1969a; Jaenike, 1978; Mousseau & Fox, 1998a) and selecting plant resources of different ages (Kearsley & Whitham, 1989).

While both host plant species and the age of plant resources can influence insect phenotypes, past studies have examined these effects in isolation, thereby missing the implications of *relative* changes in host plant species quality. Because different plant species may have unique periods of growth, maturation and senescence of tissues (i.e. unique phenology), one plant may provide superior resources for insect offspring at one point in time, while another plant species might be best at another time. Thus, the selection of host plant species by ovipositing mothers may have dynamic effects on offspring depending upon the time of year. Dynamic maternal effects may be ubiquitous in natural populations, however they have received little attention (for exceptions, see Rossiter, 1998; McAdam & Boutin, 2003; Marshall & Uller, 2007).

Maternal decisions may affect offspring at various stages of their life histories (Kerr et al., 2007); however, relatively few studies have examined effects on adult offspring reproductive traits such as mating behaviors and fecundity. These traits are often directly related to reproductive success, thus maternal effects on their expression may be especially important for offspring fitness and to population dynamics.

Here, I investigated effects of maternal host plant species choice and the timing of offspring development on adult offspring mating behaviors and fecundity in the heliconia bug, *Leptoscelis tricolor*. This work was accomplished by pairing newly-emerged males and females from two host plant species in all possible combinations and repeating this study for four sequential cohorts of insects. For each cohort I observed and recorded

mating attempts by males, female responses to these mating attempts, copulation duration, copulatory courtship behaviors, and female fecundity. I also directly examined the relationship between the phenological stage of the natal host plants and adult offspring reproductive traits.

Methods

Study species

Heliconia bugs, *Leptoscelis tricolor* (Hemiptera: Coreidae), feed, mate, and oviposit on the inflorescences of many species of heliconia plants existing in different assemblages in Costa Rica and Panama. Males and females become reproductively mature after approximately one week as an adult. Mating occurs on or near inflorescences and may last for several hours, during which time males often perform stereotyped copulatory courtship behaviors (*sensu* Eberhard, 1994) including shaking and stroking the abdomen of females with their hind legs. After mating, females immediately begin laying fertile eggs. Both adult males and females commonly fly among inflorescences and may visit multiple heliconia species during their lifetimes (see Chapter 2). Adult and juvenile insects feed on the phloem, nectar, and fruits of *Heliconia* inflorescences. Females lay eggs individually on or very near to heliconia inflorescences of various phenological stages, from inflorescences that are just beginning to bloom to those that have ripe fruits and no flowers. They may lay 200 or more eggs and live as reproductive adults 80 days or more. Total development time from egg to adult is approximately 40 days (see Chapter 2).

The location of oviposition largely determines the environment that offspring will experience during growth and development due to the limited mobility of the wingless nymphs (CW Miller, personal observation). Thus, heliconia bugs can be associated with their natal host plants simply by detecting them before they have molted into flight-capable adults. This aspect of their biology greatly facilitated extensive field sampling of large numbers of individuals.

Study procedures

Insect collection The insects in this study were detected as fifth-instar nymphs on the inflorescences of *Heliconia platystachys* and *Heliconia mariae*, growing naturally near Gamboa, Panama from June to September 2005. I enclosed each nymph and its host inflorescences within a mesh bag. If additional nymphs were present on an inflorescence, I moved them to another plant and did not include them in the experiment. When the bagged nymphs emerged as adults (up to seven days later), the mesh bags prevented them from escaping.

I returned to the bags after two weeks to collect the reproductively mature, virgin adults. The first cohort of new adults was collected on July 23, 2005, the second on August 20, 2005, the third on September 17, 2005, and the fourth on October 21, 2005. All insects in a cohort were collected within 6 hours of one another. I immediately placed collected insects individually in mesh-covered cups with moistened paper towels. They were sorted according to sex and natal host plant species and then assigned randomly to four pairing groups of all possible sex and natal host plant combinations (Table 1). 62 insects were used in each of the first three cohorts, divided nearly equally among the pairing groups. Fewer nymphs (n = 36) were available for the fourth (October) cohort, thus I eliminated groups 2 and 3 in this cohort to maintain a high sample size in groups 1 and 4. The total sample size for this study was 222 insects (111 pairs).

Table 1. Experimental study design. New adults were paired for mating based on their natal host plant species.

Pairing	Female natal host plant sp.	Male natal host plant sp.
1	*H. platystachys*	*H. platystachys*
2	*H. platystachys*	*H. mariae*
3	*H. mariae*	*H. platystachys*
4	*H. mariae*	*H. mariae*

Upon returning to the laboratory, I placed male insects into the cups of their pre-determined mates, taking approximately seven minutes to complete all pairings. Insects were kept at ambient outdoor temperatures. I observed pairs under both diffuse natural and artificial light. Previous observations indicated that light conditions do not influence copulatory and oviposition behaviors in *L. tricolor* (CW Miller, personal observation).

Copulatory behavior: After all pairs of bugs were placed together, I immediately began scan sampling the behavior of the insects, observing each pair every five minutes for four hours. I recorded if males were attempting to copulate with females, the response of females to mating attempts, and if pairs were copulating, I recorded whether copulatory courtship behaviors were being performed.

Fecundity: After adults were paired, the bugs were left in cups with ample water but without food for two days. Heliconia bugs can live without feeding for at least seven days (CW Miller, unpublished data). After two days I counted any eggs laid in the cups. All pairs in the first three cohorts were then placed in mesh bags, one pair per bag, on naturally-growing *Heliconia latispatha* inflorescences. *H. latispatha* is a common host plant for heliconia bugs in the area and was equally new to all focal individuals, making it an appropriate host for this next stage of the experiment. I selected only *H. latispatha* inflorescences with both flowers and fruits available so that insects would experience hosts of equal phenological stages. Pairs on *H. latispatha* were checked weekly and all eggs were counted and removed. Five males in the first (July) cohort died within two weeks of pairing, leaving five females without mates. I assigned these females a new mate who was raised on the same natal host plant in the same cohort as their previous mate, and I subsequently treated these pairs as all others for measures of long-term female fecundity. Egg numbers for these pairs were within one standard deviation of the average for the cohort.

Pairs in the first three cohorts were monitored for fecundity for a minimum of 23 days and a maximum of 80 days (Figure 3). All observations of the first three cohorts were terminated on October 10, 2005, and all insects were released. The final cohort was collected late in the field season (October 21, 2005), and insects were released after the mating behavior and two-day fecundity experiments. Due to slightly different care

protocols, egg counts after the first two days were not statistically compared across cohorts.

Host plant phenology: *Heliconia platystachys* and *H. mariae* differ markedly in their phenology (Croat, 1978). *H. platystachys* plants only produce inflorescences once per year, generally during the months of May and June. Over the next few months, these inflorescences progress through distinct phenological stages, until, by November, no viable inflorescences remain. *H. mariae* plants, in contrast, produce inflorescences in a staggered fashion year-round (Stiles, 1975). Thus, the different phenological schedules of these two host plant species result in striking changes in the relative resources available.

I quantitatively estimated the changing resources available for each cohort of insects by walking transect lines through patches of *Heliconia platystachys* and *H. mariae,* counting and scoring inflorescences based on the presence and condition of flowers or fruits. I determined the location of the transect line for *H. platystachys* based on the longest grid line (160m) already established in one large patch of the plant. The transect line for *H. mariae* chosen was a 2000m section of Pipeline Road in Soberania National Park with intermittent patches of *H. mariae* plants. The phenology of plants on both of these transects appeared to be representative of plants in the entire study area. I walked these transect lines each month concurrent with our searches for fifth-instar nymphs.

In addition to walking transect lines to estimate the median phenological stage of inflorescences available in the area, I also scored the stage of each inflorescence when each insect used in the experiment was collected. I used this information to compare insect reproductive traits with the median stage of inflorescences generally available and the stage of inflorescence where a particular insect was found.

Statistical analyses: I used analysis of variance to examine the effects of host plant species and month of emergence on continuous reproductive traits such as female fecundity. Logistic regression was used to examine the effects of these explanatory variables on binary response variables such as whether or not insects mated. These models included female natal host plant species, male natal host plant species, month of emergence and all two-way interactions as explanatory variables. Type IV sums-of-

squares were used to conservatively accommodate the two missing cells in the October cohort. I log-transformed continuous dependent variables when necessary to meet statistical assumptions of normality. All statistical analyses were performed with SPSS 15.0.

I next directly examined the effect of the phenological stage of individuals' natal host plant on mating probability and female fecundity. Because the median stage of *H. platystachys* inflorescences in the study area changed with time (Figure 1) the month of emergence and the phenological stage of inflorescences where individuals emerge could have both unique and correlated influences on traits. I thus partitioned variation according to these factors, based on R square values (Legendre & Legendre, 1998). This method involves partial regressions and assumes that the effects (in this case, month of emergence and phenological stage of natal inflorescence) are additive. Partial regression is a way of estimating how much of the variation of the response variable can be attributed exclusively to one set of factors, once the effect of the other set has been taken into account.

Results

Host plant phenology in the study area: The median stage of *H. platystachys* inflorescences along transect lines changed over the study period, while the median stage of *H. mariae* inflorescences remained consistent (Figure 1). Such phenological changes in *H. platystachys* parallel variation across cohorts in the probability of mating by females and also in female fecundity (see below).
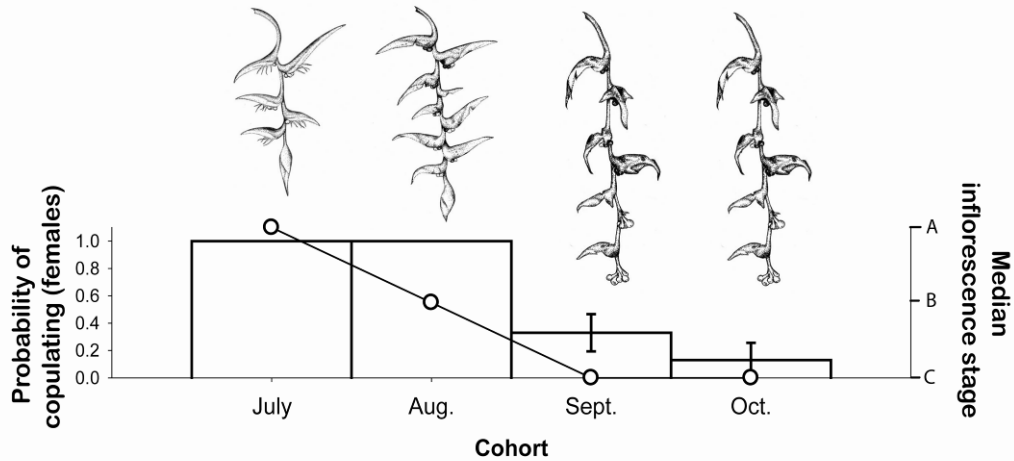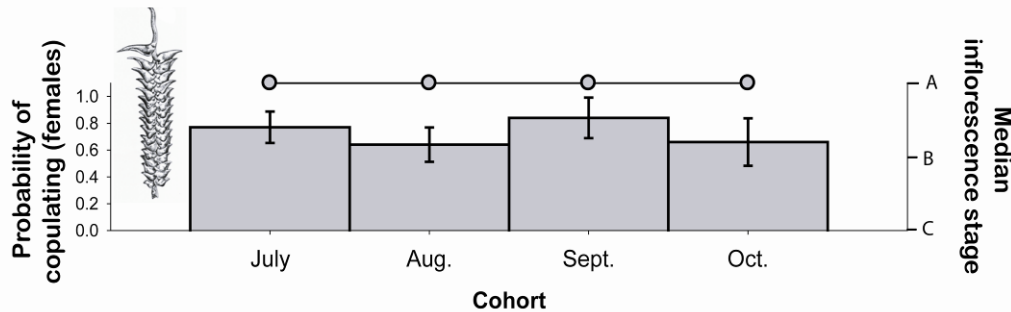
Figure 1. The probability of females copulating (bars) with courting males as related to the median inflorescence stage of heliconia host plants available in the population over time (circles). In (A) *Heliconia platystachys* inflorescences exhibited a seasonal pattern of maturation and senescence (open circles) from Stage A (both flowers and fruits available), to Stage B (only fruits available), and Stage C (inflorescence senescing). By September and October, most inflorescences were deteriorating. Females raised on these inflorescences tracked this seasonal pattern (open bars): all females emerging from *Heliconia platystachys* in the July and August cohorts copulated during our observation period, whereas females in the September and October cohorts had a lower probability of copulating. In contrast, *H. mariae* inflorescences (B) exhibited no such pattern of seasonal senescence (solid circles), and suitable inflorescences of this host species were available throughout the season. Females raised on *H. mariae* maintained a similar probability of mating across cohorts (closed bars). Drawings of heliconia illustrate representative inflorescences in each category (heliconia illustrations by David Tuss).

Mating behavior

95 of 112 (85%) females were observed receiving mating attempts from males on one or more occasions during the first four hours after pairs were formed. Neither natal host plant species of males or females nor their month of emergence (cohort) had a significant effect on which females received mating attempts and which ones did not (Table 2).

Table 2. Results of logistic regressions for mating behaviors

source	mating attempts on females		probability of mating	
	d.f.	χ^2	d.f.	χ^2
female natal species	1	< 0.01	1	7.35**
male natal species	1	0.87	1	0.01
month of emergence	1	1.61	1	0.03
female natal x month	1	0.74	1	8.15**
male natal x month	1	1.39	1	0.19
female natal x male natal	1	0.74	1	0.98

* P < 0.05, ** P < 0.02, *** P < 0.001.

In contrast, the probability of a female responding to these mating attempts by copulating with the male was significantly influenced by the natal host plant species on which she was raised (Table 2). However, this effect of natal host plant species was not consistent across cohorts (Table 2). Females from *H. platystachys* emerging in later cohorts were less likely to mate in response to male attempts than females from earlier cohorts (Figure 1). Also, the plant species producing females with the highest probability of mating switched over this time period. In earlier cohorts, females from *H. platystachys* have a higher probability of mating, while in later cohorts, females from *H. mariae* have a higher probability of mating (Figure 1). Interestingly, neither male nor female host plant species nor their month of emergence affected the duration of copulation once begun (Table 3).

Table 3. Results of analysis of variance for mating behaviors

Source	Copulation duration		Copulatory courtship start time[a]		Copulatory courtship rate[a]	
	df	*F* ratio	df	*F* ratio	df	*F* ratio
Female natal species	1	2.06	1	1.33	1	4.10*
Male natal species	1	0.38	1	2.61	1	4.10*
Month of emergence	3	1.66	1	1.92	1	2.22
Female natal x month	2	0.80	1	0.04	1	1.13
Male natal x month	2	1.83	1	0.19	1	0.33
Female natal x male natal	1	1.48	1	4.85*	1	1.21

*P < 0.05, ** P < 0.02, *** P < 0.001.*
[a] Copulatory courtship analyses include data from only July and August cohorts (see text)
Error d.f. = 56 (duration), 37 (cop. courtship start), 45 (cop. courtship rate)

Because of the strong effect of host plant species and month of emergence on female mating behavior, few copulations occurred and could be monitored for insects from *H. platystachys* in the later cohorts. I therefore analyzed copulatory courtship behaviors for only the July and August cohorts. Males in the July and August cohorts emerging from *H. platystachys* performed copulatory courtship behaviors at a faster rate than males from *H. mariae* (Table 3; Figure 2a). Furthermore, males performed a faster rate of copulatory courtship when they were paired with *females* from *H. platystachys* (Table 3; Figure 2b). The time to initiation of copulatory courtship after mating began was significantly influenced by the particular combination of males and females based on their host plant species (Table 3). In particular, when males from *H. mariae* were paired with females from *H. mariae,* copulatory courtship behaviors were initiated on average 45 minutes into copulations versus 17 minutes on average for other combinations.
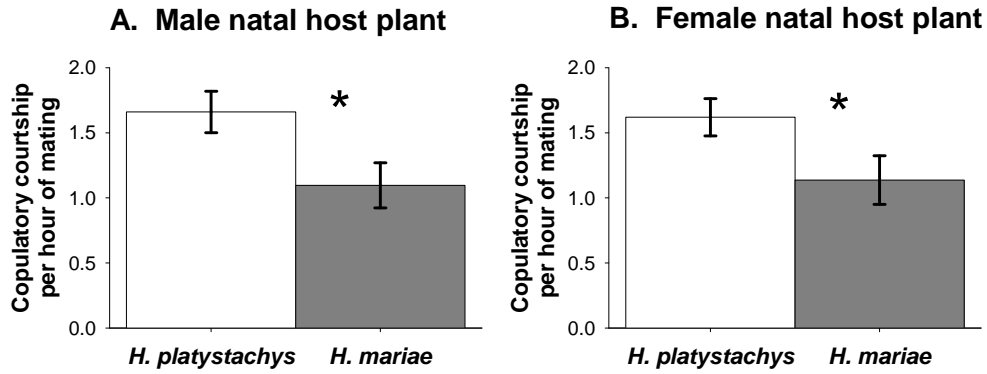
Figure 2. Means (± SE) of copulatory courtship behaviors observed per hour of mating. In *(a)* males from *H. platystachys* performed a significantly higher rate of copulatory courtship behaviors than males from *H. mariae*. In *(b)* males performed a higher rate of copulatory courtship behaviors when paired with females from *H. platystachys* than when paired with females from *H. mariae*.

<u>Fecundity</u>

Overall, females from *H. platystachys* laid more eggs than did females from *H. mariae* during the first two days after insects were paired (ANOVA: $F_{1,98} = 15.042$, $p < .001$). However, the effect of natal host plant species on initial fecundity changed dramatically across cohorts (ANOVA: $F_{2,98} = 6.649$, $p = .002$; Figure 3b) in tandem with the seasonal changes in *H. platystachys* inflorescences. Females from *H. platystachys* that emerged in July laid on average over seven times more eggs than females from *H. mariae* (means $\pm$ SE for females from *H. platystachys*: 11.40 ± 1.185 eggs versus 1.57 ± 1.37 eggs for females from *H. mariae*; Figure 3b). However, by the October cohort, immediate fecundities of females raised on *H. platystachys* had dropped to 0. At this point in the season, females from *H. mariae* laid significantly more eggs than did females from *H. platystachys* (mean $\pm$ SE for females from *H. mariae*: 4.10 ± 1.14, while none of the 8 females from *H. platystachys* produced any eggs; Figure 3b).

The initial fecundity differences between *H. platystachys* and *H. mariae* females in the July and August cohorts dissipated over time as female insects continued to lay eggs, and all were confined on the same species of plant. By day 30 of egg-laying, differences between females from *H. platystachys* and *H. mariae* were no longer statistically significant (ANOVA: $F_{1,56} = .371$, $p = .545$; Figure 3a), and total female fecundities were not different when cumulated over periods longer than 30 days ($p > .4$).
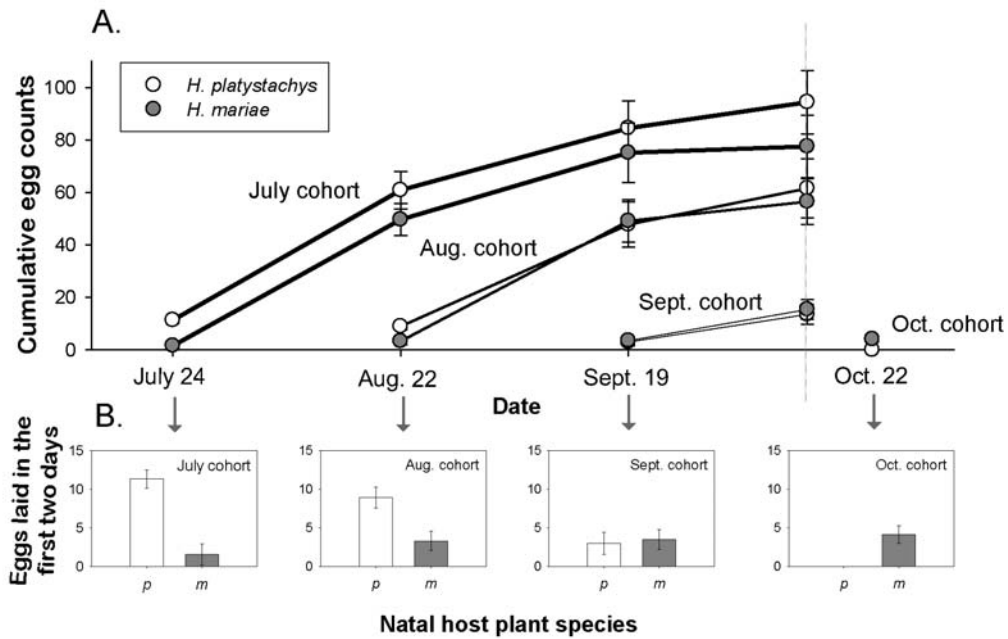
Figure 3. Means ($\pm$ SE) of the numbers of eggs produced by females in all four cohorts. In (B) females from *H. platystachys* in July and August laid significantly more eggs during the first two days after being paired. This pattern reverses, and by October, females emerging from *H. mariae* laid more eggs initially. Initial differences in egg production of females emerging in the same cohort (B) did not translate into long-term effects on fecundity (A). The dotted line indicates when (October 10th) insects from the first three cohorts were released.

<u>Host plant phenology for individual insects</u>

The stage of the *Heliconia platystachys* inflorescence where females were found as fifth-stage nymphs predicted whether females would mate following male mating attempts (means $\pm$ SE for probability of mating: .93 $\pm$ .06 from inflorescences with flowers and fruits, .65 $\pm$ .09 from inflorescences with only fruits, and .20 $\pm$ .18 from deteriorating inflorescences with only moldy fruits; logistic regression with inflorescence stage as explanatory variable: $\chi_1^2 = 7.027$, $p = .03$). *H. platystachys* inflorescence stage also predicted the initial fecundity of females (mean $\pm$ SE for inflorescences with flowers and fruits: 10.45 $\pm$ 2.02 versus 5.77 $\pm$ 1.12 for inflorescences with only fruits and .80 $\pm$ .80 for deteriorating inflorescences with only moldy fruits; ANOVA: $F_{2,48} = 4.803$, $p = .01$). Variance partitioning on *H. platystachys* female mating probability and initial egg counts suggests that the month of emergence and the phenology of individuals' host plants have shared, but also unique effects. Interestingly, the month of insect emergence had larger and more unique influences on female reproductive status than did the phenology of individual host plants. The inflorescence stage of *H. platystachys* did not significantly predict copulatory courtship start time and rate ($p > .15$). The inflorescence stage of *H. mariae* was not a significant predictor of mating behaviors and fecundity ($p > .30$), perhaps partially as a consequence of the low variability in *H. mariae* inflorescence stage in this study. 92% of insects from *H. mariae* came from inflorescences with flowers and fruits available.
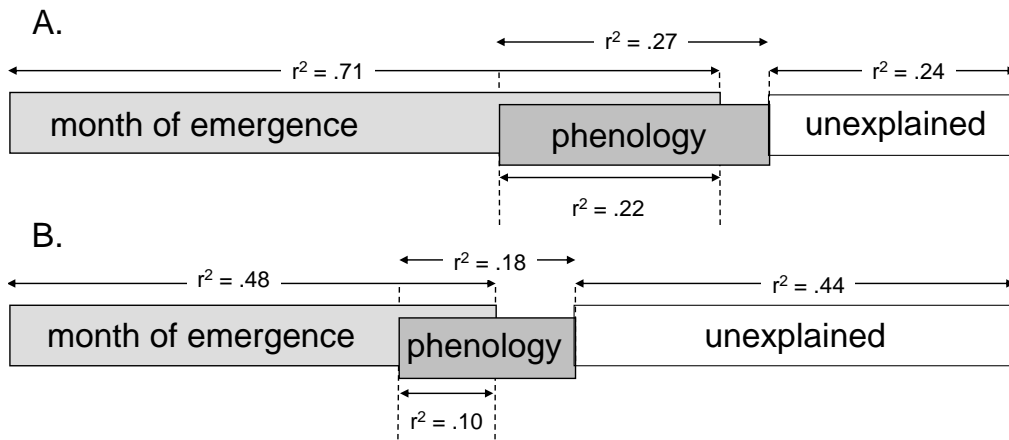
Figure 4. Partition of the variation of (A) the probability that females will copulate with receptive males, and (B) the number of eggs laid in the first two days after pairing, for females from *H. platystachys* according to the month of emergence and the phenological stage of the host plant. The length of the three boxes together corresponds to 100% of the variation in each trait. The variation explained by the phenology of natal host plants largely overlapped with month of emergence: the overlap in (A) is $r^2 = .22$ and in (B) is $r^2 = .10$. Variation in (A) was based on Nagelkerke R square values for logistic regressions, and variation in (B) was based on R square values taken from ANOVA (analysis methods based on Legendre & Legendre 1998).

<u>Discussion</u>

In this study, I found significant maternal effects in heliconia bugs, *Leptoscelis tricolor,* on multiple reproductive traits in both sons and daughters. The maternal selection of host plant species influenced offspring copulatory courtship behaviors and had large, dynamic effects over time on daughter mating probability and fecundity.

Copulatory courtship

The level of investment of a particular male in a reproductive event is likely determined by a suite of genetic and environmental factors. In this study, I examined whether the performance of copulatory courtship was shaped by: 1) the natal environment of the male himself (which may, for example, influence the amount of energy he has stored for reproductive behaviors) and 2) the natal environment of his mate (which may influence her fecundity potential and resource value to a male).

I found evidence for both of these phenomena. Males from *H. platystachys* performed copulatory courtship at a higher rate than did males from *H. mariae* (Figure 2a). In addition, males performed a higher rate of copulatory courtship when paired with females from *H. platystachys* (Figure 2b). Thus, the oviposition site selection by mothers may not only alter the copulation behaviors of their sons but also the copulation behaviors of males mating with their daughters.

Son host plant species and copulatory courtship behaviors

The broad recognition of copulatory courtship as a common and important phenomena has occurred only recently (Eberhard, 1991; Eberhard, 1994; Eberhard, 1996). Performance of copulatory courtship may have sizeable implications for male fitness (Otronen, 1990; Edvardsson & Arnqvist, 2000; Watson & Lighton, 1994; Schäfer & Uhl, 2002; Tallamy et al., 2003; Tallamy et al., 2002). For example, female cucumber beetles preferentially use the sperm of males who perform a high rate of copulatory courtship. The rate of copulatory courtship in cucumber beetles is condition-dependent and heritable (Tallamy et al., 2003). While copulatory courtship may have a heritable component in many species, my study demonstrates that it can also be significantly influenced by

environmental factors. When female heliconia bugs selected *H. platystachys* as a host plant for their offspring early in the season, their sons performed copulatory courtship at a higher rate (Table 3; Figure 2a). Such effects of mothers on the reproductive traits of sons may be common and have important implications for our understanding of sexual selection (Miller & Moore, 2007).

Daughter host plant species and copulatory courtship behaviors

The host plant choice of mothers also influenced the rate of copulatory courtship performed by males mating with their daughters (Figure 2b). Males performed copulatory courtship at a faster rate when paired with females from *H. platystachys*. Across taxa, males are expected to invest more heavily in a copulation when females are likely to have greater reproductive output (Bonduriansky, 2001). Indeed, females from *H. platystachys* in these cohorts did have significantly greater initial fecundity than did females from *H. mariae* (Figure 3; see also below). Males may have been able to perceive the reproductive potential of females, possibly through cues such as the size of the female abdomen, and in response, they may have modulated their copulatory courtship behavior. Alternatively, male copulatory courtship rate may have been the cause of female fecundity differences. This scenario is unlikely, because the rate of copulatory courtship received and the number of eggs produced by females were not directly correlated (Spearman rank correlation: $r = -.029$, $P = .875$).

Female reproductive traits

Host plant species selection by mothers influenced female offspring mating probability and initial egg production. Females emerging in July and August from *H. platystachys* had a higher probability of being receptive to male mating attempts than females emerging from *H. mariae*. However, this maternal effect reversed over time. Later cohorts of females from *H. platystachys* were much less likely to copulate than were females from *H. mariae* (Table 2; Figure 1). These patterns mirror the patterns observed in the number of eggs produced within two days after pairing (Figure 3). Females emerging in July from *H. platystachys* laid over seven times more eggs on average over these first two days than females from *H. mariae*. These patterns gradually shifted until,

in the October cohort, females from *H. mariae* laid more eggs than females from *H. platystachys*.

Initial differences in egg production did not translate into long-term, cumulative fecundity differences for females from *H. platystachys* and *H. mariae* in this study (Figure 3). Could high initial egg production have advantages for female fitness? Unlike the insects in this study, wild insects not protected by mesh bags are likely to experience high rates of predation from birds, lizards, spiders, and ants (CW Miller, personal observation). Thus, producing large quantities of offspring quickly could have fitness advantages. Females that delay reproduction or produce fewer eggs initially may die before "catching up" to females with high initial fecundity.

Given the potential fitness costs of delaying reproduction, why did females that emerged in later months on *H. platystachys* invest so little in copulation and egg laying? Certainly, variation in host plant quality might be responsible for the differences among females. Females in the later cohorts developed on older inflorescences which may have provided lower quality food, preventing females from acquiring sufficient resources for high initial egg production. Indeed, as plant tissues age, correlated changes in the levels of carbon, nitrogen, and defensive metabolites are known to influence the fecundity of insect herbivores (Awmack & Leather, 2002). In this species, the particular inflorescence stage where females from *H. platystachys* emerged was a significant predictor of mating probability and initial egg production.

The seasonal decline in female reproductive status may have been caused by a seasonal decline in resource quality, but it may also reflect a change in female reproductive strategies. While these explanations are not mutually exclusive, the changing phenology of *Heliconia platystachys* suggests that females emerging in September and October might do best to adopt a migratory phenotype. Females emerging from *H. mariae*, on the other hand, encounter a much more consistent resource over this time period (Figure 1). I elaborate on these patterns below.

Within a single *H. mariae* patch, inflorescences are commonly available year-round, with plants producing them in a staggered fashion (see also Stiles, 1975). Each month that I walked transects, the majority of inflorescences on these transect lines were producing both flowers and fruits (Figure 1). While inflorescences eventually senesce,

other, younger, inflorescences are often available within a few meters. Thus, females emerging in different cohorts will always have viable inflorescences nearby for oviposition. Based on these ecological observations, females raised on this host plant species should be predicted to be relatively constant in reproductive status across cohorts, and they are (Figure 1).

In contrast, a patch of *Heliconia platystachys* provides only ephemeral resources to heliconia bugs. Inflorescences bloom in the months of May and June. Over the next few months they go through distinct phenological stages (Figure 1). By October, most plants in the population have dropped their brown, rotten inflorescences, and all resources for these insects have effectively disappeared from the area until the next year. Thus, females emerging in September and October are surrounded by older, dying inflorescences (Figure 1). Laying eggs on dying inflorescences may result in high offspring mortality (CW Miller, personal observation), small offspring of poor condition (CW Miller, in preparation), or offspring with reduced reproductive potential (Figures 1, 3). Therefore, females developing later in the season should presumably allocate resources differently, away from current reproduction and instead to traits that will facilitate migration to viable heliconia inflorescences which can be kilometers away. Reproduction is delayed in the migratory forms of many female insects (reviewed in Zera & Denno, 1997)

Further evidence that changes in female reproductive status over time reflect different reproductive strategies comes from examining the environmental factors that best explain variation in these traits. A female's reproductive status was associated more strongly with her month of emergence than the stage of the particular *H. platystachys* inflorescence where she developed (Figure 4). Juvenile females may use olfactory or other predictable cues of general inflorescence quality in the resource patch to determine their developmental trajectories. Such broad cues of inflorescence quality in the area would be most useful to females because they commonly use multiple inflorescences for oviposition.

The association of reproductive status with month of emergence could have other explanations. It is possible that *H. platystachys* inflorescences change over time in a manner that I did not fully detect in my qualitative assessments of phenological stage.

The quality of food available, access to that food, or the quality of other resources may not be directly correlated with phenological status. Also, the month of emergence was assigned unambiguously, while scoring inflorescence stages may have a greater error component, thus weakening the statistical relationship. Regardless, changes in female reproductive traits over time suggest intriguing possibilities for further research.

Conclusions

Understanding the importance of maternal effects for the expression and evolution of offspring traits requires an appreciation of the ecological context where these effects occur. Here I have examined the effects of maternal and other environmental variation in the heliconia bug, *Leptoscelis tricolor,* on offspring reproductive traits. Female heliconia bugs select among heliconia host plant species for oviposition, and in doing so, they shape the reproductive traits of offspring. However, the relative effects of host plant selection on offspring are not consistent over time. As patches of plant resources naturally mature and senesce, the development of adult offspring phenotypes varies accordingly.

Reproductive traits are crucial to fitness, and their particular expression can have important ecological and evolutionary consequences. Increasing evidence suggests that maternal traits commonly affect the reproductive traits of adult offspring (Awmack & Leather, 2002; Clark & Galef, 1995; Doody et al., 2004; Herrenkohl, 1979; Lindström, 1999; Schluter & Gustafsson, 1993), but more work is needed. Here, I have shown that reproductive traits can be extremely environmentally labile and largely under maternal control.

When maternal effects are present, the rate and even the direction of evolution may be affected (Falconer, 1965; Kirkpatrick & Lande, 1989; Lande & Kirkpatrick, 1990; Riska et al., 1985; Wade, 1998; Cheverud & Moore, 1994; Carter et al., 2004; McAdam & Boutin, 2004; Roff, 1997). In addition, population dynamics may also be shaped by maternal effects (Bernardo, 1996; Beckerman et al., 2002; Ginzburg, 1998; Inchausti & Ginzburg, 1998; Benton et al., 2005; Smith et al., 2006). Understanding the ubiquity of maternal effects on offspring reproductive traits and how these effects

translate into ecological and evolutionary dynamics remains an important challenge for the future.

CHAPTER 6
A POTENTIAL RESOLUTION TO THE LEK PARADOX THROUGH INDIRECT GENETIC EFFECTS[1]

Abstract

Females often prefer males with elaborate traits, even when they receive no direct benefits from their choice. In such situations, mate discrimination presumably has genetic advantages; selective females will produce offspring of higher genetic quality. Over time, persistent female preferences for elaborate secondary-sexual traits in males should erode genetic variance in these traits, eventually eliminating any benefits to the preferences. Yet, strong female preferences persist in many taxa. This puzzle is called the lek paradox and raises two primary questions: do females obtain genetic benefits for offspring by selecting males with elaborate secondary-sexual characteristics, and, if so, how is the genetic variation in these male traits maintained? We suggest that indirect genetic effects may help resolve the lek paradox. Maternal phenotypes such as habitat selection behaviours and offspring provisioning often influence the condition and expression of secondary-sexual traits in sons. These maternal influences are commonly genetically based (i.e. they are indirect genetic effects). Females choosing mates with elaborate traits may receive "good genes" for daughters in the form of effective maternal characteristics. Recognizing the significance of indirect genetic effects may be important to our understanding of the process and consequences of sexual selection.

Introduction

Across many taxa, individuals go to remarkable lengths to select mates. Mate preferences are often costly, requiring time, energy, and even risk of death (Wickman & Jansson, 1997; e.g. Hedrick & Dill, 1993; Byers et al., 2005). However, the fitness benefits of choosing a good mate presumably outweigh these costs. Recognizing the benefits gained by mate choice decisions is crucial to our understanding of the process of sexual selection.

Females in many species discriminate among males based on the relative expression of secondary-sexual characteristics and often do not appear to receive any direct benefits for these mate choice behaviours. This situation is most clearly seen in lek mating systems, where females approach a group of males (i.e. a lek), select a mate, and leave with nothing but sperm. Because females receive no direct resources from mates, many researchers have surmised that these females must benefit from mate choice behaviours by obtaining genetic benefits, i.e. "good genes," for their offspring. Although female preference for good genes is intuitively appealing, and would explain the observations of mate choice without direct benefits to females, population geneticists have argued that this idea may be problematic. Robertson's corollary of Fisher's fundamental theorem (Robertson, 1966; see also Crow, 2002) suggests that over time directional selection should erode the genetic variation for secondary-sexual traits, so that females will no longer profit from discriminating among males based on these traits, and such female preferences should eventually disappear. Yet, females continually display strong preferences for males with relatively elaborate traits. This situation has been called the "lek paradox" (Kirkpatrick & Ryan, 1991; Borgia, 1979; Taylor & Williams, 1982). Many researchers have explored the lek paradox and several have proposed promising resolutions (Table 1). While the lek paradox describes only those situations where females do not receive direct, resource-based benefits from their mates (Andersson, 1994), resolving the lek paradox will have broad implications across mating systems.

Table 1: Some proposed resolutions to the lek "paradox" (Borgia 1979; Taylor & Williams 1982).

proposed resolution	references*
No cost to mate choice coupled with hidden non-genetic benefits	Reynolds & Gross 1990
Higher mutational input and selection for modifiers as a result of prolonged directional selection	Pomiankowski & Møller 1995
Genic capture through condition dependent expression of traits	Rowe & Houle 1996
Lower variance in male mating success than expected	Lanctot *et al.* 1997
Mechanisms of sexual selection result in apparent balancing selection	Moore & Moore 1999
Genotype-by-environment interactions (Context-dependent mate choice)	David *et al.* 2000; Jia *et al.* 2000
Mistake-prone mate choice	Randerson *et al.* 2000
Multivariate genetic variation actually orthogonal to direction of sexual selection	Hine *et al.* 2004

*these studies each provide an idea or model that proposes to explain how genetic variation in sexually selected traits might be maintained in systems where males provide only genetic benefits to their offspring. There are a considerable number of empirical studies that have then tested these ideas.

Of the many potential resolutions to the lek paradox, the "genic capture hypothesis" proposed by Rowe and Houle (1996), has received the most attention in recent years. This idea is based on two criteria: secondary-sexual traits exhibit condition dependence, and condition has high additive genetic variance. Secondary-sexual traits are expected to "capture" some of the additive genetic variance for condition (Rowe & Houle, 1996; Tomkins et al., 2004). Indeed, existing evidence suggests that secondary-sexual traits are commonly condition dependent (e.g. Andersson, 1994; Johnstone, 1995; Cotton et al., 2004b) and condition often has additive genetic variance (e.g. Rowe & Houle, 1996; David et al., 2000; Kotiaho et al., 2001). Therefore, this hypothesis may be an important step towards resolving the lek paradox, based on the logic that females choosing mates with elaborate traits are selecting males with "good genes" that can be passed onto offspring. However, the primary genetic influence on condition is often assumed to be *direct* additive genetic variance, i.e. genetic variation based within the focal generation. While direct additive genetic variance is undoubtedly important, it is often not a primary determinant of among-individual variation in condition (e.g. Griffith et al., 1999; Jia et al., 2000; Qvarnström, 1999).

Here we extend the ideas of Rowe and Houle to include the capture of *indirect* genetic variance. Indirect genetic effects occur when genes expressed by one individual (in this case, the mother) have phenotypic effects in another individual (in this case, the offspring; Cheverud & Moore, 1994; Moore et al., 1997; Wolf et al., 1997, 1999). Our idea is simple: mothers often influence offspring environments, and variation among mothers in their influences can be genetically based. Offspring condition is often extremely sensitive to environmental influences (e.g. Cotton et al., 2004b; Garant et al., 2004; Griffith et al., 1999) including those from mothers (Qvarnström & Price, 2001; Mousseau & Fox, 1998b). Thus, males with elaborate secondary-sexual traits might not honestly indicate superior additive genetic factors *directly* contributing to their condition, as is often envisioned. Instead, they may be signalling additive genetic factors *indirectly* influencing their condition – factors from their mothers. Females choosing males with elaborate secondary-sexual traits may be receiving "good genes" for daughters in the form of effective parenting characteristics.

In this paper we will first discuss the condition dependence of many secondary-sexual traits and the importance of the environment to phenotypic variation in condition. We will next argue that offspring environments are often shaped by their mothers, and these maternal effects may commonly have a genetic basis (i.e. they are indirect genetic effects). We present an adaptation of a previously published model (Wolf et al., 1997) to illustrate our arguments and to facilitate future empirical and theoretical studies. Finally, we discuss possible mechanisms for maintaining genetic variation in indirect genetic effects. Indirect genetic benefits from mate choice decisions may provide an important resolution to the lek paradox.

Condition and the environment

Condition dependence is a key element of many sexual selection models (e.g. Andersson, 1982; Nur & Hasson, 1984; Darwin, 1874; Fisher, 1915; West-Eberhard, 2003; Zahavi, 1975; Zahavi, 1977). Secondary-sexual traits, such as ornaments and weapons, are expected to show strong condition dependence because these traits are important to fitness, yet costly to produce. Because of the large costs involved, only those individuals of good condition will be able to produce the most elaborate traits (Andersson, 1982; Kodric-Brown & Brown, 1984; Nur & Hasson, 1984; Zeh & Zeh, 1988).

In common parlance, 'condition' is often used to indicate the general health and vigour of an individual. However, to clarify predictions and assumptions, studies of condition dependence generally define condition more narrowly as the quantity of resources available for allocation to fitness-related traits, including secondary-sexual characteristics (Lorch et al., 2003; Tomkins et al., 2004). Examining correlations between estimates of individual condition with measurements of secondary-trait expression is one way of investigating the condition dependence of secondary-sexual traits for a particular group of animals (David et al., 2000; Kotiaho et al., 2001; Parker & Garant, 2004). However, attempting to quantify condition should be undertaken with great care. Proxies for condition are often necessary because condition itself is difficult to measure directly. These proxies may or may not actually represent condition,

depending on the species, environment, and even life stage of an individual (Cotton et al., 2004b; Tomkins et al., 2004; Hunt et al., 2004). However, useful proxies for condition may be deduced with a good understanding of the acquisition, storage, and expenditure of resources by the particular research organism (Tomkins et al., 2004).

Some studies of the condition dependence of secondary-sexual traits skirt the problems of attempting to estimate condition. In these studies, researchers manipulate environmental variables experienced by groups of animals, assume that individual condition is affected in the process, and then examine the relative expression of secondary-sexual characteristics among the groups (Cotton et al., 2004b). These methods have been quite useful in determining whether secondary-sexual traits of particular species are condition dependent, and they also inadvertently underscore an important quality of individual condition – that it is easily controlled by environmental forces.

Studies from a wide range of taxa show that the quality of environments experienced often has a large and even overwhelming influence on condition and the expression of secondary-sexual traits (Ashley et al., 1998; Kaňuščák et al., 2004; Lindström, 1999; Metcalfe & Monaghan, 2001; Partridge & Endler, 1987; Post et al., 1999; Schmidt et al., 2001; Solberg & Sæther, 1994; Garant et al., 2004; Jensen et al., 2006; Griffith et al., 1999; Cotton et al., 2004b; Qvarnström, 1999). For example, males of the field cricket, *Gryllus campestris,* use a long-range song to attract females, and carrier frequency and chirp rate of these songs are reliable indicators of male condition. Males that have encountered better nutritional environments in the laboratory have lower carrier frequencies and faster chirp rates (Scheuber et al., 2003a; Scheuber et al., 2003b) . Adult carrier frequency reflects juvenile condition, while chirp rate indicates adult condition. Females prefer males exhibiting lower carrier frequencies and faster chirp rates, and they rank males with low carrier frequencies, i.e. those who experienced good nutrition when young, over males with fast chirp rates, i.e. those who experienced good nutrition when adults (Scheuber et al., 2004). In many species, when females use secondary-sexual traits to select males for copulations, they may be more likely to choose mates who have experienced high quality *environments* than males of top genetic quality. The implications of this undoubtedly common phenomenon have rarely been explored (for exceptions, see David et al., 2000; Jia et al., 2000).

What do secondary-sexual traits actually signal?

In species where males only provide sperm, females should only display strong preferences for males in good condition when condition provides an honest signal of genetic quality. While condition is often largely shaped by the environment, some environmental factors may be predictable and genetically influenced (Moore et al., 1998; Cheverud & Moore, 1994; Moore et al., 1997). Parents, and mothers in particular, can have a tremendous influence on offspring environments (Mousseau & Fox, 1998b). For instance, mothers influence offspring both prenatally and postnatally through provisioning of food, growth factors, and hormones, through selection of offspring habitat, and by protecting young (Cheverud & Moore, 1994; Mousseau & Fox, 1998a). In many species, the mother's phenotype is the single most important environmental factor encountered by an individual during development (Mousseau & Fox, 1998b). Maternal influences on offspring may be especially powerful when environments are generally stressful and offspring have limited access to resources (Carter et al., 2004; McAdam & Boutin, 2003).

Maternal effects are defined as influences of maternal phenotype on offspring phenotype, beyond the direct genetic contribution (Mousseau & Fox, 1998a). If these influences have a genetic basis in the mother, they constitute a type of indirect genetic effect. When indirect genetic effects are present, three main components contribute to an offspring's phenotype, 1) additive genetic effects, 2) non-genetic environmental effects, and 3) indirect genetic effects (see equation 4 below, Figure 1, Wolf et al., 1998).

Advantages of mating with a male who received greater maternal investment

As a twist to traditional good-genes models of sexual selection, secondary-sexual traits might serve as honest indicators of the genetic contributions to condition via maternal effects. Elaborate traits may signal the genetically-based quality of parenting that a particular male experienced. A female choosing to mate with an elaborate male may produce daughters with "good *parenting* genes" (Figure 2).
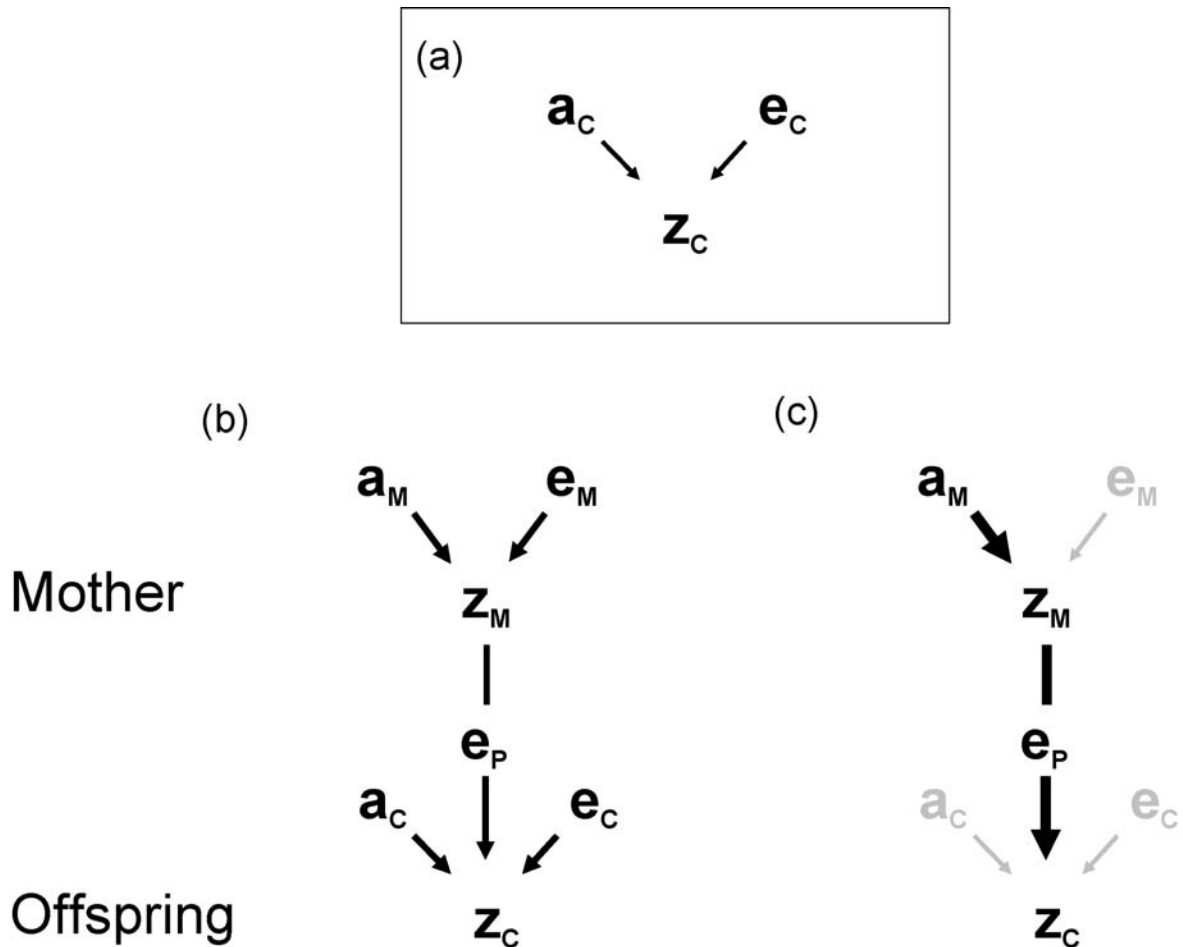
Figure 1. Diagram illustrating additive genetic (*a*) and environmental (*e*) contributions to phenotypes. In (a), the condition of an individual (z_C) reflects only additive genetic factors inherited from both parents and environmental influences. In (b), a phenotype of the mother (z_M), acts as an environmental influence (e_P, the maternal performance) on the condition of offspring (z_C). Panel (c) illustrates indirect genetic effects influencing offspring condition. Here, offspring condition (z_C) is largely determined by the environment provided by mothers (e_P). The maternal effect is itself disproportionately influenced by additive genetic factors (a_M). Modified from Moore *et al.* 1998.

GENERATION

Favourable maternal effect, z_M — P1 (mother)

e_P ⋮ a_M ↓

F1 (son)

a_M ↓

Favourable maternal effect, z_M — F2 (granddaughter)

e_P ⋮ a_M ↓

F3 (great-grandson)
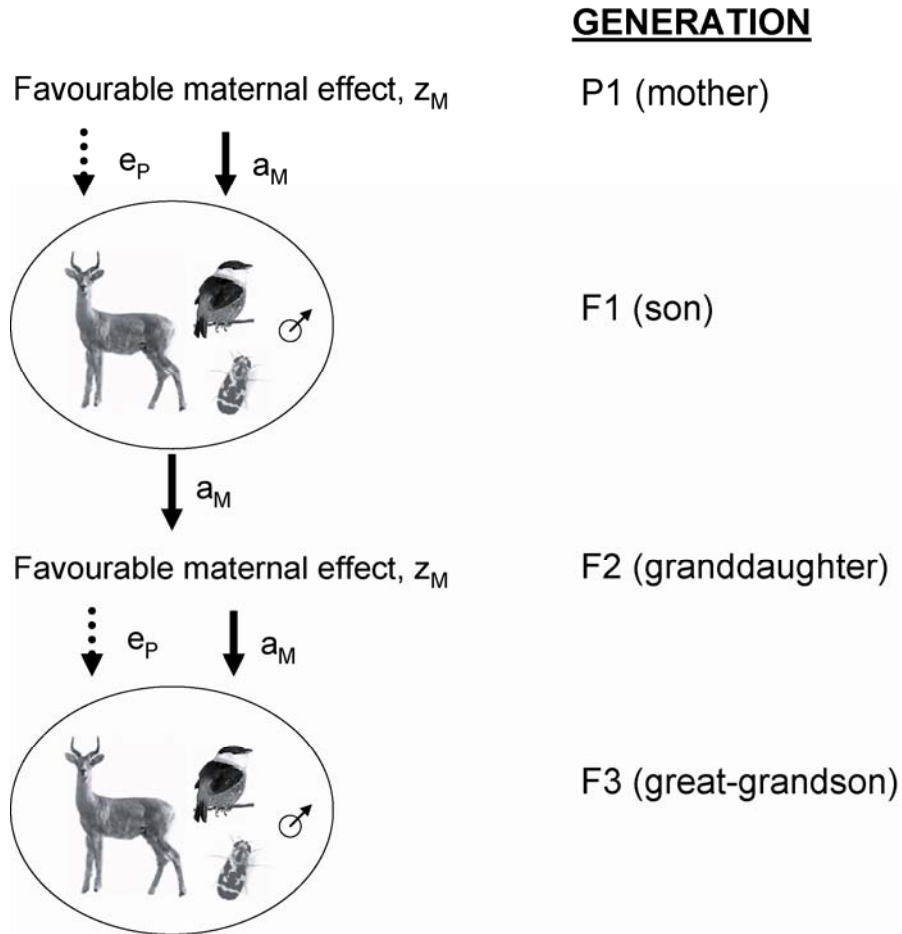
Figure 2. A hypothetical illustration of indirect genetic effects on secondary-sexual traits across four generations in three species that appear to have lek mating systems (the Uganda kob, white bearded manakin, and *Drosophila grimshawi*). In the P1 generation, a mother expresses characteristics (z_M) which result in a favourable maternal effect (e_P), on the condition of offspring. Sons in the F1 generation have not only received favourable parenting or other maternal effects, they may also obtain "good genes" (a_M) for maternal characteristics when these characteristics have an additive genetic basis. While males may not express these genes themselves, their elaborate secondary-sexual traits may signal the potential genetic benefits for their daughters (the F2 generation). Because of the maternal source of additive genetic variance of the expression of condition and secondary-sexual traits, phenotypes may "skip" generations.

The lek paradox only describes situations where males do not provide resources to females and offspring. In these mating systems males may inherit and pass on genetic information for parenting, but not express these genes themselves. While the lek paradox is best explored in such mating systems, insights into the lek paradox and possible indirect genetic benefits to mate choice might be gained by examining species with other mating systems. For instance, in the collared flycatcher, *Ficedula albicollis,* the size of the white forehead patch of males is a condition-dependent trait that predicts mating success (Griffith & Sheldon, 2001; Gustaffson et al., 1995; Pärt & Qvarnström, 1997; Qvarnström, 1999). Maternal effects, including early timing of reproduction and smaller clutch sizes, are important for the production of elaborately ornamented sons (Qvarnström, 1999). Highly significant components of variation in laying date and clutch size are attributable to additive genetic factors when measured in nature (Sheldon et al., 2003). Thus, indirect genetic effects likely play an important role in determining forehead patch size. Patch size, in turn, may signal these indirect genetic effects to potential mates. Female collared flycatchers mating with males with large forehead patches should produce daughters with maternal investment characteristics similar to their paternal grandmothers. To our knowledge this prediction has not yet been tested. While females in this species may obtain both direct, resource-based benefits from mates as well as genetic benefits for their offspring, the long-term, extensive pedigree data gathered from this species may provide an opportunity to specifically examine genetic benefits of mate choice.

The ubiquity of maternal influences on offspring phenotypes suggests that indirect genetic effects could be important players in the evolutionary process. However, more empirical studies of indirect genetic effects and their involvement in sexual selection are needed and will be facilitated by a formal framework to estimate the connections between indirect genetic effects, condition, and the expression of secondary-sexual indicator traits. To this end, we adapt a previously published model to illustrate potential relationships among these variables. Ultimately, empirical research will be needed to determine the relative importance of direct and indirect genetic effects on sexually selected characters.

A model of mate choice for condition dependent indicator traits

Wolf *et al*. (1997) modelled how indirect genetic effects arising from maternal or paternal care can directly influence a secondary-sexual indicator trait and result in mate choice for good parents. Here we consider the case when maternal performance influences offspring condition, a potentially more common occurrence. We model maternal performance, recognizing that researchers are not always able to identify the specific maternal traits that are influencing condition. Our approach assumes that maternal performance in one generation does not influence maternal performance in another generation (Cheverud & Moore, 1994; Falconer, 1965). Wolf *et al*. (1997) follow Kirkpatrick and Lande (1989) and relax this assumption, but their approach has the disadvantage of requiring that all maternal effects are known and measured. Thus, a further advantage of considering maternal performance is that maternal effects can be manipulated experimentally without knowing their specific cause through, for example, cross fostering. This approach also facilitates the quantification of maternal performance. See Cheverud and Moore (1994) for further discussion of the distinction between the models and the merits of each, as well as descriptions of methods for measuring maternal performance.

We define the phenotypic expression of three traits: offspring condition z_C, maternal performance z_M and a secondary-sexual indicator trait z_I. Maternal performance effects are assumed to vary among mothers due to genetic variation among mothers; i.e., are heritable environmental effects. Following Rowe and Houle (1996) we argue that condition (z_C) will be positively correlated with the indicator trait (z_I) such that the correlation $r_{C,I} > 0$ and when $r_{C,I} = 1$ they are the same trait. Thus, the correlation $r_{C,I}$ measures the strength of honesty of signalling condition by an indicator trait. Maternal performance effects, if present, contribute to condition rather than the indicator trait directly.

Assuming standard Mendelian rules of inheritance, and assuming polygenic influences on these continuous traits, we can define the phenotypes of these traits in terms of their additive genetic (*a*) plus environmental and non-additive genetic (*e*) components:

$$z_I = a_I + e_I \tag{5.1}$$

$$z_M = a_M + e_M \tag{5.2}$$

Condition is slightly more complicated. As we have argued above, offspring condition is expected to be influenced by maternal performance as well as genetic and other environmental effects. Thus, the model of the phenotype "condition" includes maternal effects:

$$z_C = a_C + e_C + z^*_{M_{t-1}} \tag{5.3}$$

where the sub-subscript t-1 indicates traits expressed in the previous generation (and those lacking t-1 are expressed in the current generation), and the asterisk indicates that the individuals have survived selection in that generation (Arnold, 1994). This equation highlights the distinction between different views of the contributions of additive genetic variance to condition (see also Figure 1). Often, direct (within- individual) additive genetic variance for condition is predicted to be significant to condition, z_C, and any indirect genetic effects on condition are ignored (Figure 1a). Here, we emphasize that indirect genetic effects from parents can have an important influence on condition, z_C (Figure 1c, Figure 2).

To obtain the standard quantitative genetic description of a phenotype influenced by maternal performance, we substitute for the maternal performance trait, z_M, using the equations above.

$$z_C = a_C + e_C + (a_{M_{t-1}} + e_{M_{t-1}}) \tag{5.4}$$

Central to honest-indicator models of sexual selection is the correlation between the secondary-sexual indicator trait (e.g. patch size in collared flycatchers) and condition, which can be described as

$$r_{C,I} = \frac{P_{CI}}{\sqrt{P_{CC} + P_{II}}} \qquad (5.5)$$

where $P_{i,j}$ is the phenotypic variance (when i=j) or covariance.

To consider the contributions of indirect genetic effects, we use the equations for the phenotypes above, and solve for variances and covariances under standard quantitative genetic assumptions (Lynch & Walsh, 1998)

$$P_{II} = G_{II} + E_{II} \qquad (5.6)$$

assuming no covariance between the environments of parents and offspring, and no genotype-environment covariances. The phenotypic variance for condition is more complex, because it includes covariances between direct genetic and maternal effects:

$$P_{CC} = G_{CC} + E_{CC} + \left(G_{MM} + E_{MM} + G_{CM} \right) \qquad (5.7)$$

Here, we further assume random mating with respect to maternal and paternal performance within the same generation. The phenotypic covariance between the indicator trait and condition is:

$$P_{CI} = G_{CI} + E_{CI} + \frac{G_{MI}}{2} \qquad (5.9)$$

Thus, the strength of the phenotypic correlation between condition and the indicator trait ($r_{C,I}$) in the offspring generation is influenced by direct and indirect genetic effects, including the covariance between genes for maternal performance and genes for condition, and the covariance between environmental influences on condition and environmental influences on the indicator trait. Equations (5.5-5.8) show that a genetic correlation between any aspect of maternal performance and the indicator will result in an honest signal.

What maintains additive genetic variance for maternal phenotypes?

The lek paradox raises two primary questions: do females receive genetic benefits from discriminating among males, and, if so, how is genetic variation for male traits maintained? We have argued that indirect genetic effects provide one possible source of genetic benefits to selective females. In these situations, directional selection pressures from females on males could result in the erosion of genetic variation for maternal phenotypes, favouring only those mothers who produce male offspring in good condition. However, empirical studies frequently reveal high additive genetic variance and moderate to high heritability in maternal phenotypes or their influences on offspring (e.g. Freeman-Gallant & Rothstein, 1999; Sheldon et al., 2003; Hunt & Simmons, 2002; MacColl & Hatchwell, 2003; McAdam et al., 2002; Rauter & Moore, 2002; Van Tassell et al., 1999; Wilson et al., 2005; Merilä & Sheldon, 2000). How is this genetic variation in maternal phenotypes maintained? Evolutionary biologists recognise that various mechanisms may be responsible for maintaining additive genetic variance in the face of what appear to be strong directional selection pressures (Falconer & Mackay, 1996; Roff, 1997). Here, we will highlight two potential general mechanisms that might be particularly important in maintaining additive genetic variance for maternal characteristics.

Work by Randerson *et al.* (2000) suggests that genetic variation for an elaborate secondary-sexual trait can be maintained indefinitely due to occasional mistakes made by females in discriminating among potential mates (Neff, 2000). Thus, the directional selection for maternal characteristics that produce sons with elaborate traits may not be as strong as we initially suspect. Mistakes in mate choice may be inevitable, due to the environmental sensitivity of condition in males. Unpredictable or infrequent environmental variation may occasionally obscure maternal influences on the condition of males. In addition, females may occasionally select sub-optimal males (who received sub-optimal parenting) because of the likely costs involved in finding the best mates, competing for mates, or accurately assessing male quality (e.g. Dale et al., 1992; Byers et al., 2005; Godin & Briggs, 1996; Hedrick & Dill, 1993; Neff, 2000; Randerson et al., 2000). In some cases, female searching behaviour and mate assessment may themselves

be condition dependent, with females of poor condition unable or unwilling to invest in finding the highest-quality mates (Burley & Foster, 2006; Hunt et al., 2005). Work on flycatchers suggest that females may be socially or otherwise constrained in their selection of mates, and thus mate choice "mistakes" in this species may be common (Dale et al., 1992; Qvarnström et al., 2006).

Genotype-by-environment (GEI) interactions are another related mechanism that could explain the maintenance of additive genetic variance in maternal phenotypes. Previous work on GEI suggests that they, combined with environmental heterogeneity and gene flow (Gillespie & Turelli, 1989), may be important in maintaining genetic variance in many traits (Shaw et al., 1995; Sultan & Bazzaz, 1993; Charmantier & Garant, 2005) including secondary-sexual characteristics (Jia et al., 2000; Rodriguez & Greenfield, 2003; Welch, 2003; Qvarnström, 1999). GEI of mothers may result in maternal genotypes that are successful in one environment (producing attractive offspring of good condition) being relatively unsuccessful in another environment (producing unattractive offspring of poor condition). A small amount of gene flow between populations where different reaction norms are favoured is all that is needed to maintain additive genetic variance in maternal characteristics.

The maintenance of genetic variation is one of the main unresolved issues in evolutionary biology, and many other mechanisms may be important. To most appropriately address this issue, researchers must understand the *source* of the additive genetic variation contributing to phenotypes. Previous studies addressing the maintenance of additive genetic variation in condition have generally focused on *direct* additive genetic variation, even though it may only responsible for a small proportion of the total phenotypic variation in this trait. Future studies must also consider the maintenance of genetic variance in maternal phenotypes.

Conclusions

Mothers often differ significantly in the type and intensity of their influences on offspring, and these differences can have striking implications for offspring phenotypes (Clutton-Brock, 1991). High additive genetic variance and moderate to high heritabilities

for maternal characteristics appear to be common (Freeman-Gallant & Rothstein, 1999; Sheldon et al., 2003; Hunt & Simmons, 2002; MacColl & Hatchwell, 2003; McAdam et al., 2002; Merilä & Sheldon, 2000; Rauter & Moore, 2002; Van Tassell et al., 1999; Wilson et al., 2005), and indirect genetic effects may create complex evolutionary dynamics. For instance, theoretical work suggests that traits with little or no direct additive genetic variance can still evolve if indirect genetic effects are present (Cheverud & Moore, 1994; Moore et al., 1997). Additionally, when both direct genetic variance and indirect genetic variance contribute to a trait, the response to selection may be accelerated, slowed, or even in the opposite direction from predicted (Kirkpatrick & Lande, 1989; McAdam & Boutin, 2004). An increasing number of researchers are recognizing the importance of examining the genetic basis behind maternal effects in natural situations (e.g. McAdam et al., 2002; Wilson et al., 2005; Sheldon et al., 2003). Further empirical research is needed to fully understand the implications of indirect genetic effects for evolutionary processes.

We suggest that indirect genetic effects may help resolve the lek paradox. When secondary-sexual traits are tightly associated with condition, and condition is significantly influenced by indirect genetic effects, females who choose males with elaborate secondary-sexual traits may obtain "good genes" for their daughters in the form of effective maternal characteristics. Strong preferences by females for males with elaborate traits could result in the erosion of genetic variation for maternal phenotypes, favouring only those mothers who produce male offspring in good condition. However, additive genetic variance for maternal characteristics appears to be common, and could be maintained by a variety of mechanisms which have yet to be empirically explored in this context. By incorporating our understanding of the condition-dependent nature of secondary-sexual traits with investigations of indirect genetic effects, we may find important answers to the long-standing enigma of why males express such elaborate traits and why females prefer them.

BIBLIOGRAPHY

Aldrich, J. R. & Blum, M. S. 1978. Aposematic aggregation of a bug (Hemiptera-Coreidae) - defensive display and formation of aggregations. *Biotropica*, **10,** 58-61.

Andersson, M. 1982. Sexual selection, natural selection and quality advertisement. *Biological Journal of the Linnean Society*, **17,** 375-393.

Andersson, M. 1994. *Sexual Selection*. Princeton: Princeton University Press.

Arnold, S. J. 1994. Multivariate inheritance and evolution: a review of concepts. In: *Quantitative Genetics Studies of the Evolution of Behavior* (Ed. by Boake, C. R. B.), 17-48. Chicago: University of Chicago Press.

Ashley, E. P., McCullough, G. B. & Robinson, J. T. 1998. Morphological responses of white-tailed deer to a severe population reduction. *Canadian Journal of Zoology*, **76,** 1-5.

Awmack, C. S. & Leather, S. R. 2002. Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47,** 817-844.

Beckerman, A., Benton, T. G., Ranta, E., Kaitala, V. & Lundberg, P. 2002. Population dynamic consequences of delayed life-history effects. *Trends in Ecology and Evolution*, **17,** 263-269.

Benton, T. G., Plaistow, S. J., Beckerman, A. P., Lapsley, C. T. & Littlejohns, S. 2005. Changes in maternal investment in eggs can affect population dynamics. *Proceedings of the Royal Society - Biological Sciences (Series B)*, **272,** 1351-1356.

Bernardo, J. 1996. Maternal effects in animal ecology. *American Zoologist*, **36,** 83-105.

Blatt, S. E. & Borden, J. H. 1996. Evidence for a male-produced aggregation pheromone in the western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae). *Canadian Entomologist*, **128,** 777-778.

Bonduriansky, R. 2001. The evolution of male mate choice in insects: A synthesis of ideas and evidence. *Biological Reviews of the Cambridge Philosophical Society*, **76,** 305-339.

Bonduriansky, R. 2007. The evolution of condition-dependent sexual dimorphism. *American Naturalist*, **169**, 9-19.

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

Borgia, G. 1979. Sexual selection and the evolution of mating systems. In: *Sexual Selection and Reproductive Competition in Insects* (Ed. by Blum, M. S. & Blum, N. A.), 19-80. New York: Academic Press.

Brittingham, M. C. & Temple, S. A. 1996. Vegetation around parasitized and non-parasitized nests within deciduous forest. *Journal of Field Ornithology*, **67,** 406-413.

Burley, N. T. & Foster, V. S. 2006. Variation in female choice of mates: condition influences selectivity. *Animal Behaviour*, **72,** 713-719.

Byers, J. A., Wiseman, P. A., Jones, L. & Roffe, T. J. 2005. A large cost of female mate sampling in pronghorn. *American Naturalist*, **166,** 661-668.

Carroll, S. C. 1980. Reproductive and developmental biology of *Chelinidea vittiger* Uhler (Hemiptera: Coreidae). M.S. Thesis. Lubbock: Texas Tech University.

Carter, M. J., Lardies, M. A., Nespolo, R. F. & Bozinovic, F. 2004. Heritability of progeny size in a terrestrial isopod: Transgenerational environmental effects on a life history trait. *Heredity*, **93,** 455-459.

Caut, S., Guirlet, E., Jouquet, P. & Girondot, M. 2006. Influence of nest location and yolkless eggs on the hatching success of leatherback turtle clutches in French Guiana. *Canadian Journal of Zoology*, **84,** 908-915.

Charmantier, A. & Garant, D. 2005. Environmental quality and evolutionary potential: Lessons from wild populations. *Proceedings of the Royal Society: Biological Sciences (Series B)*, **272,** 1415-1425.

Cheverud, J. M. & Moore, A. J. 1994. Quantitative genetics and the role of the environment provided by relatives in behavioral evolution. In: *Quantitative genetic studies in behavioral evolution* (Ed. by Boake, C. R. B.), 67-100. Chicago: University of Chicago Press.

Clark, M. M. & Galef, B. G. 1995. Prenatal influences on reproductive life history strategies. *Trends in Ecology and Evolution*, **10,** 151-153.

Clutton-Brock, T. H. 1991. *The evolution of parental care*. Princeton, N.J.: Princeton University Press.

Conner, J. K. & Hartl, D. L. 2004. *A primer of ecological genetics*. Sunderland, MA: Sinauer Associates, Inc.

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

Cotton, S., Fowler, K. & Pomiankowski, A. 2004b. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **271,** 771-783.

Cotton, S., Fowler, K. & Pomiankowski, A. 2004c. Heightened condition dependence is not a general feature of male eyespan in stalk-eyed flies (Diptera: Diopsidae). *Journal of Evolutionary Biology*, **17,** 1310-1316.

Croat, T. 1978. *Flora of Barro Colorado Island*. Stanford, CA: Stanford University Press.

Crow, J. F. 2002. Here's to Fisher, additive genetic variance, and the fundamental theorem of natural selection. *Evolution*, **56,** 1313-1316.

Dale, S., Rinden, H. & Slagsvold, T. 1992. Competition for a mate restricts mate search of female pied flycatchers *Behavioral Ecology and Sociobiology*, **30**.

Darlington, R. B. & Smulders, T. V. 2001. Problems with residual analysis. *Animal Behaviour*, **58,** 1261-1267.

Darwin, C. R. 1874. *The descent of man and selection in relation to sex*. New York: Hurst and Company.

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

David, P., Hingle, A., Greig, D., Rutherford, A., Pomiankowski, A. & Fowler, K. 1998. Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **265,** 2211-2216.

David, P., Moreteau, B., Gauthier, J. P., Petave, G., Stockel, A. & Imasheva, A. G. 1994. Reaction norms of sie characters in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis. *Genetics Selection Evolution*, **26,** 229-251.

DeVol, J. E. 1969. The biology of *Chelinidea vittiger* Uhler, Hemiptera-Heteroptera: Coreidae in southern California. M.S. Thesis. Riverside: University of California, Riverside.

Doody, J. S., Georges, A. & Young, J. E. 2004. Determinants of reproductive success and offspring sex in a turtle with environmental sex determination. *Biological Journal of the Linnean Society*, **81,** 1-16.

Eberhard, W. G. 1991. Copulatory courtship and cryptic female choice in insects. *Biological Reviews of the Cambridge Philosophical Society*, **66,** 1-31.

Eberhard, W. G. 1994. Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. *Evolution*, **48,** 711-733.

Eberhard, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton, NJ: Princeton University Press.

Eberhard, W. G. 1998. Sexual behavior of *Acanthocephala declivis guatemalana* (Hemiptera : Coreidae) and the allometric scaling of their modified hind legs. *Annals of the Entomological Society of America*, **91,** 863-871.

Edvardsson, M. & Arnqvist, G. 2000. Copulatory courtship and cryptic female choice in red flour beetles *Tribolium castaneum. Proceedings of the Royal Society - Biological Sciences (Series B)*, **267,** 559-563.

Einum, S. & Fleming, I. A. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): Norms of reaction to environmental quality. *Proceedings of the Royal Society - Biological Sciences (Series B)*, **266,** 2095-2100.

Emlen, D. 1997. Diet alters male horn alometry in the beetle *Onthphagus acuminatus* (Coleoptera: Scarabaeidae). *Proceedings of the Royal Society - Biological Sciences (Series B)*, **264,** 567-574.

Emlen, D. J. & Allen, C. E. 2003. Genotype to phenotype: Physiological control of trait size and scaling in insects. *Integrative and Comparative Biology*, **43,** 617-634.

Falconer, D. S. 1965. Maternal effects and selection response. In: *Genetics today: proceedings of the XI international congress of genetics* (Ed. by Greet, S. J.), 763-764. Oxford: Pergamon.

Falconer, D. S. & Mackay, T. F. C. 1996. *Introduction to Quantitative Genetics*. Harlow: Prentice Hall.

Fisher, R. A. 1915. The evolution of sexual preference. *Eugenics Review*, **7,** 184-192.

Freeman-Gallant, C. R. & Rothstein, M. D. 1999. Apparent heritability of parental care in Savannah Sparrows. *Auk*, **116,** 1132-1136.

Fujisaki, K. 1981. Studies on the mating system of the winter cherry bug, *Acanthocoris sordidus* Thunberg (Heteroptera, Coreidae): 2. harem defense polygyny. *Researches on Population Ecology*, **23,** 262-279.

Garant, D., Sheldon, B. C. & Gustafsson, L. 2004. Climatic and temporal effects on the expression of secondary sexual characters: Genetic and environmental components. *Evolution*, **58,** 634-644.

Garcia-Bertou, E. 2001. On the misuse of residuals in ecology: testing regression residuals versus the analysis of covariance. *Journal of Animal Ecology*, **70,** 708-711.

Gillespie, J. H. & Turelli, M. 1989. Genotype-environment interactions and the maintenance of polygenic variation. *Genetics*, **121,** 129-138.

Ginzburg, L. R. 1998. Inertial growth: population dynamics based on maternal effects. In: *Maternal Effects as Adaptations* (Ed. by Mousseau, T. & Fox, C. W.), 42-53. New York: Oxford University Press.

Godfray, H. C. J. 1995. Evolutionary theory of parent-offspring conflict. *Nature*, **376,** 133-138.

Godin, J. G. J. & Briggs, S. E. 1996. Female mate choice under predation risk in the guppy. *Animal Behaviour*, **51,** 117-130.

Griffith, S. C., Owens, I. P. F. & Burke, T. 1999. Environmental determination of a sexually selected trait. *Nature*, **400,** 358-360.

Griffith, S. C. & Sheldon, B. C. 2001. Phenotypic plasticity in the expression of sexually selected traits: neglected components of variation. *Animal Behaviour*, **61,** 987-993.

Gustaffson, L., Qvarnström, A. & Sheldon, B. C. 1995. Trade-offs between life history traits and a secondary sexual character in male collared flycatchers. *Nature*, **375,** 311-313.

Hartwig, E. K. & De Lange, H. C. 1978. Observations on three coreids, *Carlisis wahlbergi* Stal, *Anoplocnemis curvipes* (Fabr.) and *Elasmopoda valga* (L.). In: *Technical Communication* (Ed. by Department of Agricultural Technical Services, R. o. S. A.), 11 pages: Government Printer.

Hedrick, A. V. & Dill, L. M. 1993. Mate choice by female crickets is influenced by predation risk. *Animal Behaviour*, **46,** 193-196.

Herrenkohl, L. R. 1979. Prenatal stress reduces fertility and fecundity in female offspring. *Science*, **206,** 1097-1099.

Hingston, R. W. G. 1929. A study in insect protection (*Anoplocnemis phasiana*, FABR.). *Journ Bom Nat Hist Soc*, **33,** 341-346.

Hunt, J., Brooks, R. & Jennions, M. D. 2005. Female mate choice as a condition-dependent life-history trait. *American Naturalist*, **166,** 79-92.

Hunt, J., Bussière, L. F., Jennions, M. D. & Brooks, R. 2004. What is genetic quality? *Trends in Ecology and Evolution*, **19,** 329-333.

Hunt, J. & Simmons, L. W. 2002. The genetics of maternal care: direct and indirect genetic effects in the dung beetle *Onthophagus taurus*. *Proc. Natl Acad. Sci. USA*, **99,** 6828-6832.

Inchausti, P. & Ginzburg, L. R. 1998. Small mammal cycles in northern Europe: Patterns and evidence for a maternal effect hypothesis. *Journal of Animal Ecology*, **67,** 180-194.

Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. *Theoretical Population Biology*, **14,** 350-356.

Janz, N., Nylin, S. & Wedell, N. 1994. Host plant utilization in the comma butterfly: sources of variation and evolutionary implications. *Oecologia*, **99,** 132-140.

Jensen, H., Svorkmo-Lundberg, T., Ringsby, T. H. & Sæther, B. E. 2006. Environmental influence and cohort effects in a sexual ornament in the house sparrow, *Passer domesticus*. *Oikos*, **114,** 212-224.

Jia, F. Y., Greenfield, M. D. & Collins, R. D. 2000. Genetic variance of sexually selected traits in waxmoths: Maintenance by genotype x environment interaction. *Evolution*, **54,** 953-967.

Johnstone, R. A. 1995. Honest advertisement of multiple qualities using multiple signals. *Journal of Theoretical Biology*, **177,** 87-94.

Kaňuščák, P., Hromada, M., Tryjanowski, P. & Sparks, T. 2004. Does climate at different scales influence the phenology and phenotype of the River Warbler *Locustella fluviatilis*? *Oecologia*, **141,** 158-163.

Karan, D., Morin, J.-P., Gilbert, P., Moreteau, S. M., Scheiner, S. M. & David, J. R. 2000. The genetics of phenotypic plasticity. IX. Genetic architecture, temperature, and sex differences in *Drosophila melanogaster*. *Evolution*, **54,** 1035-1040.

Katvala, M. & Kaitala, A. 2003. Conspecifics enhance egg production in an egg-carrying bug. *Behavioral Ecology*, **14,** 897-901.

Kearsley, M. J. C. & Whitham, T. G. 1989. Developmental changes in resistance to herbivory: implications for individuals and populations. *Ecology*, **70,** 422-434.

Kerr, T. D., Boutin, S., LaMontagne, J. M., McAdam, A. G. & Humphries, M. M. 2007. Persistent maternal effects on juvenile survival in North American red squirrels. *Biology Letters*, **3,** 289-291.

Kirkpatrick, M. & Lande, R. 1989. The evolution of maternal characters. *Evolution*, **43,** 485-503.

Kirkpatrick, M. & Ryan, M. J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature*, **350,** 33-38.

Kodric-Brown, A. & Brown, J. H. 1984. Truth in advertising: the kinds of traits favored by sexual selection. *American Naturalist*, **124,** 309-323.

Kolbe, J. J. & Janzen, F. J. 2002. Impact of nest-site selection on nest success and nest temperature in natural and disturbed habitats. *Ecology*, **83,** 269-281.

Kotiaho, J. S., Simmons, L. W. & Tomkins, J. L. 2001. Towards a resolution of the lek paradox. *Nature*, **410,** 684-686.

Lande, R. & Kirkpatrick, M. 1990. Selection response in traits with maternal inheritance. *Genetical Research*, **55,** 189-197.

Leal, W. S., Panizzi, A. R. & Niva, C. C. 1994. Alarm pheromone system of leaf-footed
bug *Leptoglossus zonatus* (Heteroptera: Coreidae). *Journal of Chemical Ecology*,
20, 1209-1216.

Legendre, P. & Legendre, L. 1998. *Numerical Ecology*. Amsterdam: Elsevier.

Lindström, J. 1999. Early development and fitness in birds and mammals. *Trends in
Ecology and Evolution*, **14,** 343-348.

Lorch, P. D., Proulx, S., Rowe, L. & Day, T. 2003. Condition-dependent sexual selection
can accelerate adaptation. *Evolutionary Ecology Research*, **5,** 867-881.

Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sunderland,
MA: Sinaeur Associates.

MacColl, A. D. C. & Hatchwell, B. J. 2003. Heritability of parental effort in a passerine
bird. *Evolution*, **57,** 2191-2195.

Marshall, D. J. & Uller, T. 2007. When is a maternal effect adaptive? *Oikos*, **116,** 1957-
1963.

Martin, T. E. 1992. Interaction of nest predation and food limitation in reproductive
strategies. *Current Ornithology*, **9,** 163-197.

Martin, T. E., Scott, J. & Menge, C. 2000. Nest predation increases with parental activity:
Separating nest site and parental activity effects. *Proceedings of the Royal Society
- Biological Sciences (Series B)*, **267,** 2287-2293.

Mayhew, P. J. 1997. Adaptive patterns of host-plant selection by phytophagous insects.
Oikos, **79,** 417-428.

Mayhew, P. J. 2001. Herbivore host choice and optimal bad motherhood. *Trends in
Ecology and Evolution*, **16,** 165-167.

McAdam, A. G. & Boutin, S. 2003. Effects of food abundance on genetic and maternal
variation in the growth rate of juvenile red squirrels. *Journal of Evolutionary
Biology*, **16,** 1249-1256.

McAdam, A. G. & Boutin, S. 2004. Maternal effects and the response to selection in red
squirrels. *Proceedings of the Royal Society of London, Series B: Biological
Sciences*, **271,** 75-79.

McAdam, A. G., Boutin, S., Réale, D. & Berteaux, D. 2002. Maternal effects and the
potential for evolution in a natural population of animals. *Evolution*, **56,** 846-851.

Merilä, J. & Sheldon, B. C. 2000. Lifetime reproductive success and heritability in nature. *American Naturalist*, **155,** 307-310.

Metcalfe, N. B. & Monaghan, P. 2001. Compensation for a bad start: Grow now, pay later? *Trends in Ecology and Evolution*, **16,** 254-260.

Miller, C. W. & Moore, A. J. 2007. A potential resolution to the lek paradox through indirect genetic effects. *Proceedings of the Royal Society - Biological Sciences (Series B)*, **274,** 1279-1286.

Mitchell, P. L. 1980. Combat and Territorial Defense of *Acanthocephala femorata* (Hemiptera, Coreidae). *Annals of the Entomological Society of America*, **73,** 404-408.

Mitchell, P. L. 2000. Leaf-footed bugs (Coreidae). In: *Heteroptera of Economic Importance* (Ed. by Schaefer, C. W. & Panizzi, A. R.), 337-402. New York: CRC Press.

Mitchell, P. L. 2006. Polyphagy in true bugs: a case study of *Leptoglossus phyllopus* (L.) (Hemiptera, Heteroptera, Coreidae). *Denisia 16, Zugleich Kataloge der OÖ. Landesmuseen Neue Serie* **50,** 1117-1134.

Moczek, A. P. & Emlen, D. J. 1999. Proximate determination of male horn dimorphism in the beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). *Journal of Evolutionary Biology*, **12,** 27-37.

Møller, A. P. & Petrie, M. 2002. Condition dependence, multiple sexual signals, and immunocompetence in peacocks. *Behavioral Ecology* **13,** 248-253.

Moore, A. J., Brodie, E. D., III & Wolf, J. B. 1997. Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution*, **51,** 1352-1362.

Moore, A. J., Wolf, J. B. & Brodie, E. D. I. 1998. The influence of direct and indirect genetic effects on the evolution of behavior: social and sexual selection meet maternal effects. In: *Maternal effects as adaptations* (Ed. by Fox, C. W. & Mousseau, T. A.), 23-41. New York: Oxford University Press.

Mousseau, T. A. & Fox, C. W. 1998a. The adaptive significance of maternal effects. *Trends in Ecology & Evolution*, **13,** 403.

Mousseau, T. A. & Fox, C. W. 1998b. Maternal Effects as Adaptations. New York: Oxford University Press.

Neff, B. D. 2000. Females aren't perfect: maintaining genetic variation and the lek paradox. *Trends in Ecology & Evolution*, **15,** 395.

Nishida, T. 1989. Scramble Competition Polygyny in the Coreid Bug (*Colpula lativentris*). *Researches on Population Ecology*, **31,** 211-224.

Nur, N. & Hasson, O. 1984. Phenotypic plasticity and the handicap principle. *Journal of Theoretical Biology*, **110,** 275-297.

Nylin, S. & Janz, N. 1996. Host plant preferences in the comma butterfly (*Polygonia c-album*): do parents and offspring agree? *Ecoscience*, **3,** 285-289.

Otronen, M. 1990. Mating behavior and sperm competition in the fly, *Dryomyza anilis*. *Behavioral Ecology & Sociobiology*, **26,** 349-356.

Pakkasmaa, S., Merila, J. & O'Hara, R. B. 2003. Genetic and maternal effect influences on viability of common frog tadpoles under different environmental conditions. *Heredity*, **91,** 117-124.

Parichy, D. M. & Kaplan, R. H. 1992. Maternal effects on offspring growth and development depend on environmental-quality in the frog *Bombina orientalis*. *Oecologia*, **91,** 579-586.

Parker, T. H. & Garant, D. 2004. Quantitative genetics of sexually dimorphic traits and capture of genetic variance by a sexually-selected condition-dependent ornament in red junglefowl (*Gallus gallus*). *Journal of Evolutionary Biology*, **17,** 1277-1285.

Pärt, T. & Qvarnström, A. 1997. Badge size in collared flycatchers predicts outcome of male competition over territories. *Animal Behaviour*, **54,** 893-899.

Partridge, L. & Endler, J. A. 1987. Life history constraints on sexual selection. *Sexual Selection: Testing the Alternatives*, 265-277.

Post, E., Langvatn, R., Forchhammer, M. C. & Stenseth, N. C. 1999. Environmental variation shapes sexual dimorphism in red deer. *Proceedings of the National Academy of Science of the United States of America*, **96,** 4467-4471.

Price, T. & Schluter, D. 1991. On the low heritability of life-history traits. *Evolution*, **45,** 853-861.

Qvarnström, A. 1999. Genotype-by-environment interactions in the determination of the size of a secondary sexual character in the collared flycatcher (*Ficedula albicollis*). *Evolution*, **53,** 1564-1572.

Qvarnström, A., Brommer, J. E. & Gustafsson, L. 2006. Testing the genetics underlying the co-evolution of mate choice and ornament in the wild. *Nature*, **441,** 84-86.

Qvarnström, A. & Price, T. D. 2001. Maternal effects, paternal effects and sexual selection. *Trends in Ecology & Evolution*, **16,** 95-100.

Randerson, J. P., Jiggins, F. M. & Hurst, L. D. 2000. Male killing can select for male mate choice: A novel solution to the paradox of the lek. *Proceedings of the Royal Society - Biological Sciences (Series B)*, **267,** 867-874.

Rasband, W. 1997-2005. ImageJ: http://rsb.info.nih.gov/ij/. Bethesda, MD: US National Institutes of Health.

Rausher, M. D. 1980. Host abundance, juvenile survival, and oviposition preference in *Battus philenor*. *Evolution*, **34,** 342-355.

Rausher, M. D. 1981. host plant selection by *Battus philenor* butterflies: the roles of predation, nutrition, and plant chemistry. *Ecological Monographs*.

Rauter, C. M. & Moore, A. J. 2002. Quantitative genetics of growth and development time in the burying beetle *Nicrophorus pustulatus* in the presence and absence of post-hatching parental care. *Evolution*, **56,** 96-110.

Riska, B., Rutledge, J. J. & Atchley, W. R. 1985. Covariance between direct and maternal genetic effects in mice, with a model of persistent environmental influences. *Genetical Research*, **45,** 287-297.

Robertson, A. 1966. A mathematical model of the culling process in dairy cattle. *Animal Production*, **8,** 95-108.

Rodriguez, R. L. & Greenfield, M. D. 2003. Genetic variance and phenotypic plasticity in a component of female mate choice in an ultrasonic moth. *Evolution*, **57,** 1304-1313.

Roff, D. A. 1992. *The Evolution of Life Histories: Theory and Analysis*. New York: Chapman and Hall.

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

Rossiter, M. 1998. The role of environmental variation in parental effects expression. In: *Maternal effects as adaptations* (Ed. by Mousseau, T. A. & Fox, C. W.), 112-134. New York: Oxford University Press.

Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society - Biological Sciences (Series B)*, **263,** 1415-1421.

Schäfer, M. A. & Uhl, G. 2002. Determinants of paternity success in the spider *Pholcus phalangioides* (Pholcidae: Araneae): The role of male and female mating behaviour. *Behavioral Ecology and Sociobiology*, **51,** 368-377.

Scheirs, J., De Bruyn, L. & Verhagen, R. 2000. Optimization of adult performance determines host choice in a grass miner. *Proceedings of the Royal Society - Biological Sciences (Series B)*, **267,** 2065-2069.

Scheuber, H., Jacot, A. & Brinkhof, M. W. G. 2003a. Condition dependence of a multicomponent sexual signal in the field cricket *Gryllus campestris*. *Animal Behaviour*, **65,** 721-727.

Scheuber, H., Jacot, A. & Brinkhof, M. W. G. 2003b. The effect of past condition on a multicomponent sexual signal. *Proceedings of the Royal Society - Biological Sciences (Series B)*, **270,** 1779-1784.

Scheuber, H., Jacot, A. & Brinkhof, M. W. G. 2004. Female preference for multiple condition-dependent components of a sexually selected signal. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **271,** 2453-2457.

Schluter, D. & Gustafsson, L. 1993. Maternal inheritance of condition and clutch size in the collared flycatcher. *Evolution*, **47,** 658-667.

Schmidt, K. T., Stien, A., Albon, S. D. & Guinness, F. E. 2001. Antler length of yearling red deer is determined by population density, weather and early life-history. *Oecologia*, **127,** 191-197.

Scribner, J. M. & Slansky Jr. , F. 1981. The nutritional ecology of immature insects. *Annual Review of Entomology*, **26,** 183-221.

Shaw, R. G., Platenkamp, G. A. J., Shaw, F. H. & Podolsky, R. H. 1995. Quantitative genetics of response to competitors in *Nemophila menziesii*: A field experiment. *Genetics*, **139,** 397-406.

Sheldon, B. C., Kruuk, L. E. B. & Merilä, J. 2003. Natural selection and inheritance of breeding time and clutch size in the collared flycatcher. *Evolution*, **57,** 406-420.

Shingleton, A. W., Frankino, W. A., Flatt, T., Nijhout, H. F. & Emlen, D. J. 2007. Size and shape: The developmental regulation of static allometry in insects. *BioEssays*, **29,** 536-548.

Skutch, A. F. 1949. Do tropical birds rear as many young as they can nourish? *Ibis*, **91,** 430-455.

Smith, L. A., White, P. C. L. & Hutchings, M. R. 2006. Effect of the nutritional environment and reproductive investment on herbivore-parasite interactions in grazing environments. *Behavioral Ecology*, **17,** 591-596.

Solberg, E. J. & Sæther, B. E. 1994. Male traits as life-history variables: Annual variation in body mass and antler size in moose (*Alces alces*). *Journal of Mammalogy*, **75,** 1069-1079.

Stiles, F. G. 1975. Ecology, flowering phenology, and hummingbird pollination of some Costa Rican heliconia species. *Ecology*, **56,** 285-301.

Stratton, D. A. 1989. Competition prolongs expression of maternal effects in seedlings of *Erigeron annuus* (Asteraceae). *American Journal of Botany*, **76,** 1646-1653.

Sultan, S. E. & Bazzaz, F. A. 1993. Phenotypic plasticity in *Polygonum persicaria*. II. Norms of reaction to soil moisture and the maintenance of genetic diversity. *Evolution*, **47,** 1032-1049.

Sutherland, O. R. W. 1969a. The role of the host plant in the production of winged forms by two strains of the pea aphid *Acyrthosiphon pisum*. *Journal of Insect Physiology*, **15,** 2179-2201.

Sutherland, O. R. W. 1969b. The role of the host plant in the production of winged forms by two strains of the pea aphid *Acyrthosiphon pisum*. *J. Insect Physiol.*, **15,** 2179-2201.

Tallamy, D. W., Darlington, M. B., Pesek, J. D. & Powell, B. E. 2003. Copulatory courtship signals male genetic quality in cucumber beetles. *Proceedings of the Royal Society of London - B. Biological Sciences*, **270,** 77-82.

Tallamy, D. W., Powell, B. E. & McClafferty, J. A. 2002. Male traits under cryptic female choice in the spotted cucumber beetle (Coleoptera: Chrysomelidae). *Behavioral Ecology*, **13,** 511-518.

Taylor, P. D. & Williams, G. C. 1982. The lek paradox is not resolved. *Theoretical Population Biology* **22,** 392-409.

Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata*, **47,** 3-14.

Thornhill, R. & Alcock, J. 1983. *The Evolution of Insect mating Systems*. Cambridge: Harvard University Press.

Tomkins, J. L., Radwan, J., Kotiaho, J. S. & Tregenza, T. 2004. Genic capture and resolving the lek paradox. *Trends in Ecology and Evolution*, **19,** 323-328.

Valladares, G. & Lawton, J. H. 1991. Host-plant selection in the holly leaf-miner: does mother know best? *Journal of Animal Ecology*, **60,** 227-240.

Van Tassell, C. P., Wiggans, G. R. & Norman, H. D. 1999. Method R estimates of heritability for milk, fat, and protein yields of United States dairy cattle. *Journal of Dairy Science*, **82,** 2231-2237.

Wade, M. J. 1998. The evolutionary genetics of maternal effects. In: *Maternal Effects as Adaptations* (Ed. by Fox, C. W. & Mousseau, T. A.), 5-21. New York: Oxford University Press.

Wang, Q. & Millar, J. G. 2000. Mating behavior and evidence for male-produced sex pheromones in *Leptoglossus clypealis* (Heteroptera : Coreidae). *Annals of the Entomological Society of America*, **93,** 972-976.

Watson, P. J. & Lighton, J. R. B. 1994. Sexual selection and the energetics of copulatory courtship in the Sierra dome spider, *Linyphia litigiosa*. *Animal Behaviour*, **48,** 615-626.

Wedell, N. 1996. Mate quality affects reproductive effort in a paternally investing species. *American Naturalist*, **148,** 1075-1088.

Weladji, R., Holand, O., Steinheim, G., Colman, J. E., Gjostein, H. & Kosmo, A. 2005. Sexual dimorphism and intercohort variation in reindeer calf antler length is associated with density and weather. *Oecologia*, **145,** 549-555.

Welch, A. M. 2003. Genetic benefits of a female mating preference in gray tree frogs are context-dependent. *Evolution*, **57,** 883-893.

West-Eberhard, M. J. 1986. Alternative adaptations, speciation and phylogeny (a review). *Proceedings of the National Academy of Science of the United States of America*, **83,** 1388-1392.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, **20,** 249-278.

West-Eberhard, M. J. 2003. *Developmental plasticity and evolution*. Oxford: Oxford University Press.

Wickman, P. O. & Jansson, P. 1997. An estimate of female mate searching costs in the lekking butterfly *Coenonympha pamphilus. Behavioral Ecology and Sociobiology*, **40,** 321-328.

Wilson, A. J., Coltman, D. W., Pemberton, J. M., Overall, A. D. J., Byrne, K. A. & Kruuk, L. E. B. 2005. Maternal genetic effects set the potential for evolution in a free-living vertebrate population. *Journal of Evolutionary Biology*, **18,** 405-414.

Wolf, J. B., Brodie, E. D., III, Cheverud, J. M., Moore, A. J. & Wade, M. J. 1998. Evolutionary consequences of indirect genetic effects. *Trends in Ecology & Evolution*, **13,** 64-69.

Wolf, J. B., Moore, A. J. & Brodie, E. D., III. 1997. The evolution of indicator traits for parental quality: The role of maternal and paternal effects. *American Naturalist*, **150,** 639-649.

Yasuda, K. 1998. Function of the male pheromone of the leaf-footed plant bug, *Leptoglossus australis* (Fabricius) (Heteroptera : Coreidae) and its kairomonal effect. *Jarq-Japan Agricultural Research Quarterly*, **32,** 161-165.

Zahavi, A. 1975. Mate selection - A selection for a handicap. *Journal of Theoretical Biology*, **53,** 205-214.

Zahavi, A. 1977. The cost of honesty. (Further remarks on the handicap principle). *Journal of Theoretical Biology*, **67,** 603-605.

Zeh, D. W. & Zeh, J. A. 1988. Condition-dependent sex ornaments and field tests of sexual-selection theory. *American Naturalist*, **132,** 454-459.

Zera, A. J. & Denno, R. F. 1997. Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology*, **42,** 207-231.

Appendix A: Estimation of heritabilities of body size and shape in the heliconia bug, *Leptoscelis tricolor*

Background

Recent evidence suggests that secondary-sexual characteristics may have low heritabilities, especially in natural populations. Here, I conducted a full-sib quantitative genetics breeding experiment during the rainy season of 2004 to estimate the heritabilities of body and secondary-sexual traits in the heliconia bug, *Leptoscelis tricolor*. I also estimated the relative survivorship of nymphs raised on three of the most common species of heliconia host plants in the area around Gamboa, Panama.

Methods

Parents

In August, 2004 my assistants and I searched naturally-growing heliconia inflorescences near Gamboa, Panama for fifth-stage *Leptoscelis tricolor* nymphs. When we detected a nymph, we slipped a fine mesh bag over the individual and its host inflorescence and secured the bag closed. Any additional nymphs (generally 0 to 3) were removed from the inflorescence and not included in the study. When the bagged nymphs molted into adults (up to seven days later), the mesh bags prevented them from flying off. These insects achieve reproductive maturity between one week and two weeks after becoming adults. My assistants and I returned three weeks later to collect the reproductively mature new adults.

Immediately after collection, males and females were paired randomly, creating a total of twenty pairs, and placed alone in a mesh bag over an inflorescence of a naturally-growing *Heliconia platystachys*, *H. mariae,* or *H. latispatha* plant. After six days my assistants and I returned to the bags to count the number of eggs laid. If < 15 eggs were laid, we left the pairs in place for another six days for a maximum of twelve days. Pairs were then rotated to the remaining heliconia species, following the same protocol. The order of

heliconia species where pairs were placed was determined randomly, with all pairs experiencing each host plant species once. During the time period of these rotations a member of a pair commonly died or escaped, thus not all families had representatives in all heliconia host species environments.

Rearing of nymphs

My assistants and I collected eggs (n = 15, when possible) from breeding bags after the period of egg-laying and then we placed individual eggs on inflorescences of the same heliconia species on which their mothers had originally laid the eggs. Prior to egg placement, inflorescences were visually inspected and cleaned with water from a spray bottle and toothbrushes to remove any eggs present, spiders, ants, and debris. We secured mesh bags with eggs closed and monitored offspring for survivorship to adulthood. After approximately forty days, we collected new adult offspring, digitally captured images through a Leica light microscope, and used ImageJ software (Rasband, 1997-2005) to measure pronotum width and hind femur width at the third distal spine.

Statistical estimation of heritabilities

Broad sense heritabilities and standard errors were estimated from one-way ANOVAs (Becker, 1984) using data from the full-sib breeding experiment. Generally for the estimation of heritabilities only siblings raised in similar environmental conditions are compared. This procedure is used because traits often vary predictably and significantly due to environmental differences. If siblings raised in substantially different environments are compared, the environmental component of phenotypic variation will be inflated and useful information about environmental effects will be ignored (Conner & Hartl, 2004). Such across-environmental differences in trait size should be expected when heliconia bugs develop earlier in the rainy season (see results). However, this breeding experiment was conducted during the time when new adults emerging from different host plant species did not significantly differ ($P > 0.3$ in 2005) in body and leg sizes. Because there was no evidence that these plants had different environmental effects on insects, I pooled all nymphs within families for the analyses to increase the

precision of the heritability estimate. The results for pooled insects were similar to results when only full-sib insects raised on the same species of heliconia were compared.

Full-sib breeding experiments, such as I did here, are simpler in design than half-sib breeding experiments and are much more feasible in the field. However, full sib analyses may inflate heritabilities due to dominance effects. Thus, the heritability estimates here are best regarded as an upper limit estimate of heritabilities in this population.

Results and conclusions

Survivorship and general rearing outcomes

One of the great advantages of conducting breeding experiments in the field is that conditions are realistic and measurements of trait expression and heritability should be more relevant to the natural population under study. However, tradeoffs often come with attempting these large experiments under natural conditions. One tradeoff here was the low survivorship of nymphs. Of the 568 mesh bags with offspring, only 138 (24%) had nymphs that survived to adulthood. The survivorship of offspring was not equal across all host plant species. On *H. platystachys* only 21/195 (11%) survived, while 70/214 (33%) survived on *H. latispatha* and 47/159 (30%) survived on *H. mariae*. These survivorship differences across plant species may reflect the deterioration of *H. platystachys* inflorescences in the population during the month of October when nymphs were being reared (Figure 1).

Heritabilities

Surviving nymphs emerged as adults between the dates of September 29th and November 9th, 2004. While likely all traits contain some heritable genetic variation, even if it is quite low, I found no evidence for a heritable component of body or hind femur width, relative hind femur width (calculated from the residuals of the regression of leg on body size), or front femur area. Full-sib heritability analyses for male body size (measured as pronotum width), male mean hind femur width, relative leg width and front femur area were low and not significantly different from zero, suggesting that little heritable

variation exists for these traits in this population when reared under natural conditions (male body: H^2 = -0.477 $\pm$ 0.088; male hind femur width: H^2 = -0.412 $\pm$ 0.110; male relative leg: H^2 = 0.233 $\pm$ 0.252; male front femur area: H^2 = -0.176 $\pm$ 0.176; all heritability estimates $p > 0.1$, as indicated by the ANOVA F tests). Female body size, hind femur width, relative hind femur width, and front femur area also had low and non-significant heritabilities (female body: H^2 = -0.301 $\pm$ 0.131; female hind femur: H^2 = -.124 $\pm$ 0.173; female front femur area: H^2 = -.181 $\pm$ 0.161; all heritability estimates $p > 0.1$). The mean number of offspring surviving per plant species per family was 2.65 $\pm$ 0.50, and this low sample number may be one reason for the non-significant heritability estimates.

The low heritabilities in male body and leg size contrast sharply with the clear effects of natal host plant and month of emergence in shaping these traits (CW Miller, Chapters 3 & 4). Thus, in heliconia bugs, a clear understanding of the environmental influences on trait expression will be important for understanding the patterns of expression of morphological traits and their evolution.