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INFLUENCES OF HOST SIZE AND HOST QUALITY ON HOST USE IN A SEED-FEEDING BEETLE

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ABSTRACT OF DISSERTATION

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The Graduate School
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2006

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ABSTRACT OF DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
at the University of Kentucky

By
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Lexington, Kentucky
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2006

ABSTRACT OF DISSERTATION

INFLUENCES OF HOST SIZE AND HOST QUALITY ON HOST USE IN A SEED-FEEDING BEETLE

For insects that develop inside discrete hosts both host size and host quality constrain offspring growth, influencing the evolution of body size and life history traits. This dissertation examines the effects of host size, host quality, and intraspecific competition on life history and associated traits of populations of the seed-feeding beetle *S. limbatus* adapted to different host plants, and quantifies population differences in phenotypic plasticity. Populations of the study correspond to divergent clades of the species phylogeography (Colombia and United States).

Clades compared differ genetically for all traits when beetles were raised in a common garden. Contrary to expectations from the local adaptation hypothesis, beetles from all populations were larger, developed faster and had higher survivorship when reared in *Acacia greggii*, the larger host. Two host-plant mediated maternal effects were found: offspring matured sooner, regardless of their rearing host, when their mothers were reared on *Pseudosamanea guachapele* and females laid larger eggs on *Ps. guachapele*. These results also show that this species in addition to be a smaller is a low quality host. Females also laid more eggs and sooner on *A*. *greggii* than in *Ps. guachapele* and, laid more eggs on *P. guachapele* when *A. greggii* seeds were small than when they were large. Eggs were larger when laid on *Ps. guachapele* and *Parkinsonia florida*, two hosts that reduce survivorship in all populations. However, Colombia females laid eggs of similar size on *Ps. guachapele* and *Pa. florida,* while USA females laid the largest eggs on *Pa. florida*. Larger beetles were most affected when larval competition was increased and seed size decreased. The responses of different body sized females were asymmetrical showing significant variation in plasticity.

Although differences between populations in growth and life history traits appear to be adaptations to the size and quality of their host plants, host-associated maternal effects, partly mediated by maternal egg size plasticity play an important role in the evolution of *S. limbatus*' diet breadth. More generally, phenotypic plasticity mediates the fitness consequences of using novel hosts, likely facilitating colonization of new hosts but also buffering herbivores from selection post-colonization.

KEY WORDS: Local adaptation, host use, phenotypic plasticity, *Stator limbatus*, population variation

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This dissertation is dedicated to my loving husband Carlos E. Sarmiento-Monroy, my daughter, María Alejandra Sarmiento-Amarillo, my parents Celestino Amarillo and Fidelia Suárez, my guide and friend Dr. Charles W. Fox and Dr. Bobby Pass without whose encouragement and support this work would not have been possible.

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Chapter 1: Introduction

Understanding behavioral, physiological and ecological factors that influence host use by insects, and the ways in which these factors interact to produce variation at different levels (within individuals, among individuals within populations, among populations, and among species), is an important objective of the field of evolutionary ecology (Mazer and Damuth 2001). These factors may have genotypic, phenotypic and ecological bases and determine properties of host use such as host performance and host discrimination.

The interaction between organisms and their environment, in this case an insect and its host plant, is mediated by both behavioral and physiological traits. Ecological and environmental factors such as temperature, natural enemies, competition, and host quality, impose selection on insect behavior and physiology. For parasitoids and seed feeder insects, in which development occurs in a single host and for which larvae cannot move among hosts (they are restricted to the host selected by their mother), resources are limited by host size. Both host size and quality are therefore major determinants of selection on female oviposition decisions and subsequent adaptation to host plants. Host size constrains the evolution of adult size and clutch size (Hardy et al. 1992, Allen and Hunt 2001, Mackauer and Chau 2001, Tsai et al. 2001). Body size increases with host size, both among populations and species (i.e., populations and species adapted to larger hosts evolve larger body size) and within populations (individuals reared on larger hosts tend to mature at larger size; Kirk 1991), though there are exceptions to these patterns. For example, in the seed beetle *Callosobruchus maculatus,* the effect of seed size on body size is the opposite: Beetles from populations adapted to small size seeds are larger than beetles from populations adapted to large seeds. The explanation for this pattern is related to the type of competition experienced by larvae developing inside of the seed – beetles adapted to small seeds evolve contest competition, which favors large body size, whereas beetles that use large seeds evolved scramble competition (Tokenaga and Fuji 1990, Messina 1991a).

Within populations, larger hosts may support a larger number of individuals (e.g., larvae), but increased clutch size increases larval competition among co-specifics which in turn reduces the resources available to individual larvae, increasing larval mortality and because resources run out sooner, reducing larval development time and body size at maturation (Fox et al. 1996, Ode et al. 1996, Fox and Savalli 1998).

The quality of the host is influenced by defensive mechanisms in the plant such as secondary compounds, and by the nutritional value of the plant tissues. Secondary compounds may deter insect feeding or cause higher mortality. Feeding on low nutritional hosts may extend development time or even prevent normal development, increasing the susceptibility of organisms to pathogens and exposing immature to higher probability of mortality by natural enemies (Schoonhoven et al. 1998) among others. In consequence, females have evolved strategies to evaluate host quality and availability, and adjust their oviposition behavior and their resource allocation to offspring. For example, in seed-feeding beetles females avoid adding eggs to seeds already bearing eggs (Messina and Renwick 1985, Messina and Mitchell 1989, Tsai et al. 2001), adjust clutch size in response to host size (e.g., lay more eggs on larger hosts), and tend to distribute their eggs more evenly on smaller seeds (Hardy et al. 1992). However, the host that females prefer is not always the best for the performance or their progeny; female oviposition behaviors evolve in response to host abundance (Singer 1983, Jaenike 1990) and other ecological factors that influence larval mortality risks, such as susceptibility to natural enemies (Ballabeni et al. 2001, Stamp 2001).

1.1 Population Variation in Host Use: Local Adaptation and Phenotypic Plasticity

Many studies show the existence of variation in host use within populations - i.e., individuals within the same population vary in host preference and behavior to hosts of varying quality and size (Mopper et al. 1984, Thompson 1988, Fry 1992, Fox et al. 1994, Kraaijeveld et al.1995, Hess et al. 1996). Likewise, populations vary substantially in host preference and the behavior of females towards hosts of varying quality and size (Prokopy et al. 1984, Papaj 1986, Waring et al. 1990, Singer and Thomas 1996).

In general, there are two sources of variation among and within populations: genetic and environmental. These sources interact to generate the phenotypes observed in nature. The interaction between genetic and environmental sources of variation generates genetic differentiation among populations and phenotypic plasticity within populations. In the field of host selection by phytophagous insects, including behaviors such as preference for hosts and acceptability of hosts for oviposition may be variable (Bernays and Chapman 1994). Variation among populations is mainly due to genetic differentiation among populations caused by adaptation to local host species being greater than the homogenizing effect of gene flow between populations. Disruptive selection (Singer and Parmesan 1993) generates differentiation among populations in which some phenotypes are adapted to specific host characteristics, leading to local adaptation and host specialization. In contrast, individuals within the same population should have very similar patterns of host choice, oviposition preference and performance of their offspring (Jaenike 1990) because selection is expected to maintain cohesion between individuals of the same population.

This variation also affects female oviposition preference. For example, studies of the butterfly *Euphydrias editha* show that females of Del Puerto Canyon prefer oviposit upon their local host than on the host of *E. edytha* from Indian Flat, and that larvae from Del Puerto Canyon, perform better on this host than in the host of larvae from Indian Flat (Singer et al. 1988). Populations of *P. glaucus* from Georgia and Florida were tested in their oviposition preference and larval performance, finding significant genetic differentiation among populations in these traits. This difference resulted of strong selection that enhanced the use of Magnolia on the Florida population (Bossart and Scriber 1995). Numerous other studies have now shown similar degrees of adaptation to local host plants in herbivorous insects (Mopper 1996).

Intrapopulation variation is mainly due to phenotypic plasticity (the expression of different phenotypes by a given genotype; Via 1994). Thus, variation in host availability could lead to the evolution of phenotypic plasticity being an important mechanism of adaptation to variable environments (Futuyma 2001), and facilitating colonization and expansion into new environments (Fox and Savalli 2000). Plastic responses to host plants may be mediated by maternal experiences, a phenomenon called cross-generational (or trans-generational) phenotypic plasticity (Mousseau and Dingle 1991) in which parents modify the phenotype of their offspring in response to environmental conditions (Fox and Mousseau 1998, Lacey 1998). Because oviposition behavior and host choice are maternal characters the environment in which females develop and their phenotype may influence offspring performance and survivorship. For example, In the seed beetles *Stator limbatus* and *Callosobruchus maculatus* there is a strong influence of the clutch size on offspring size and development time. Large clutches per seed produce small progeny hatching from small eggs in *C. maculatus* and extension of development time to reach a normal body size in *S. limbatus* (Fox 2000b). Females may also be able to program developmental changes in offspring in response to environmental cues (Csezack and Fox 2003).

1.2 *Stator limbatus*: a system to study population variation in host use

Stator limbatus is a seed feeding beetle (Coleoptera: Chrysomelidae: Bruchinae) with a broad distribution in the Americas. Populations are found from the northwest of Argentina to the southwest in the United States (Johnson and Kingsolver 1976, Johnson et al. 1989). This species lives mostly in desert or semiarid environments and use ≥ 80 host plant species in at least 9 genera throughout its broad distribution. Although *S. limbatus* is considered a generalist because of the large number of hosts it uses, host use varies substantially among localities and most populations use few hosts and are thus specialists relative to the diversity of plant species available to them (Fox et al. 1995, Morse and Farrell 2005a, b).

Females of *S. limbatus* oviposit directly onto the mature seeds of their hosts. After hatching, the larvae burrow into, and develop completely inside, the seed. Beetles emerge from seeds as adults and start oviposition 12-48 h later. The complete life cycle takes 28-30 days at 28^{o}C.

Studies previously done with this beetle have shown local adaptation of populations differing in their native host. For example, the Scottsdale population (Arizona) collected from *Parkinsonia florida* and the Black Canyon population (Arizona) collected from *Acacia greggii* differ in preference for host seeds and have higher fecundity and perform better on their native host. The differences in survivorship and development time observed between these populations represent genetic differences in the ability of populations to use *P. florida* and are also mediated by maternal effects (Fox et al. 1994). Similar results have been shown using other populations differing in their native host plant (Fox et al. 1997).

Given that this beetle develops completely inside a single seed, and is unable to move among seeds, the physiological and ecological factors that mediate adaptation to host seeds will be directed largely to overcome hurdles to access and use individual seeds; among those hurdles are seed size, seed quality, and intra and interpopulation competition. Seed composition of the hosts that populations of *S. limbatus* use vary, imposing substantial variation in selection on individuals using these species. Because host use varies among populations, this variation in selection favors local adaptation and thus substantial differentiation among populations that use different host species. For example, populations from Arizona use as their major hosts seeds of *Acacia greggii* and *Parkinsonia florida* which differ in the chemical composition of their seed coats. *A. greggii* seeds do not have toxic substances in their seed coat and thus larvae reared on these seeds have high survival, regardless of egg size. This allows females to lay small eggs, and thus have high fecundity, when their larvae will develop on *A. greggii* seeds. In contrast, seed coats of *P. florida* seeds are largely resistant to penetration by beetle larvae, and there is a large effect of egg size on larval survival (larvae from large eggs are better able to penetrate seed coats than are larvae from small seeds). This imposes selection on egg size and females have responded by evolving egg size plasticity in which they lay larger eggs on seeds of *P. florida* (and necessarily have lower fecundity) than when ovipositing on seeds of *A. greggii* (on which they lay smaller eggs and have higher fecundity) (Fox et al. 1999). Thus, when comparing selection across the three host species commonly used by *S. limbatus* in the southwestern United States, there is intense selection for egg size when eggs are laid on *P. florida*, intermediate selection on *P. microphylla* and very low selection on *A. greggii* (Fox 2000a). Females respond to this by laying larger eggs on *P. florida* than on either *P. microphylla* or *A. greggii* (Fox et al. 2001).

A second major source of selection on beetles is mediated through seed size. In *S. limbatus*, populations from Colombia adapted to the small seeds of *Pseudosamanea guachapele* are around 40 to 50% smaller than populations adapted to the large seeds of *A. berlandieri* (Populations from Texas, USA) (Figure 1.1). Studies using populations from Arizona have shown that seed size has significant effects on adult body size. Thus, beetles raised on large seeds are larger and have longer development time than beetles that developed in small seeds (Fox et al. 1996).

Seed size also influences clutch size and female preferences. In the congeneric seed beetle *S. beali*, females laying eggs on a mixed treatment containing a large and a small seed of their native host, *Chloroleucon ebano,* preferred to lay eggs on the large seed and, when forced to lay eggs only on a seed of a specific size, females adjusted clutch size in response to seed size (Fox and Mousseau 1995). In nature seeds are a limiting resource for *S. limbatus* such that females are forced to lay several eggs per seed, and oviposit on seeds bearing eggs laid by other females. Larval competition within seeds results in beetles maturing smaller than beetles emerging from seeds with fewer eggs (Fox 1997b).

Because *S. limbatus* larvae cannot move among seeds, it is expected that seed size and seed quality will have important consequences for life history and associated traits. In addition, populations of *S. limbatus* are adapted to different host plants making possible the comparison of life histories among them. However, there are no studies analyzing population variation in response to seed size and quality on *S. limbatus*, and no work has been done comparing plastic responses to these factors among populations located outside of the United States nor using hosts other than *A. greggii* and *P. florida* (which produce seeds that are quite similar in size). Populations used through this study correspond to very divergent clades of *S. limbatus*. Colombia populations are located into the South American clade and the United States populations are included in the North American clade. This phylogenetic divergence has a genetic basis strongly influenced by the reduction in gene flow between populations across geographic barriers (Morse and Farrell 2005a). These barriers are, from south to north, The Andes Mountains in South America, the Isthmus of Panama and the Sierra Madre Oriental and Cordillera Transvolcanica in Mexico.

The goal of my research in this dissertation is to (a) examine the effects of host size, host quality, and intraspecific competition on life history and associated traits on populations of *S. limbatus* adapted to different host plants and (b) to quantify population differences in phenotypic plasticity in response to host size, host quality, and intraspecific competition. This dissertation includes three major projects: In the first I examined the contributions of local adaptation, phenotypic plasticity and maternal effects to differences in growth and life history traits between populations adapted to hosts that differ in size and quality (Chapter 2). In the second project I examined the influence of host size, host species (quality), and body size on host discrimination, oviposition behavior and egg size (Chapter 3). Finally, I examined how host size, host quality and beetle body size influence the consequences of larval competition for growth and life history traits of *S. limbatus* (Chapter 4).

Figure 1.1: Differences in body size among beetles of: (a) Del Rio population (Texas, USA) and, (b) Anapoima (Cundinamarca, Colombia).

Chapter 2: Population differences in host use: Local adaptation, phenotypic plasticity and maternal effects

2.1 Introduction

Variation among host plants is an important determinant of phenotypic variation in herbivorous insects (Ohsaki and Sato 1994, Mira and Bernays 2002, Singer and Stireman 2003). Species for which host availability varies among populations may become genetically differentiated due to adaptation to their local hosts (Mopper 1996 and references therein). When trade-offs in host use exist, local adaptation can come at a cost of decreased performance on alternative hosts (Van Zandt and Mopper 1998, Agrawal 2000). Variation in host availability could also result in the evolution of phenotypic plasticity in which the same genotype expresses different phenotypes on different hosts (Via 1994). Phenotypic plasticity can be an important mechanism of adaptation to variable environments (Futuyma 2001), can facilitate colonization and expansion into new environments (Fox and Savalli 2000), and may even influence the evolution of community structure by molding multitrophic interactions (Agrawal 2001). Plastic responses to host plants may be mediated by maternal experiences, a phenomenon called cross-generational (or trans-generational) phenotypic plasticity (Mousseau and Dingle 1991) in which parents modify the phenotype of their offspring in response to environmental conditions (Fox and Mousseau 1998, Wade 1998, Lacey 1998, Mazer and Damuth 2001); e.g., mothers may program developmental changes in their offspring, or change patterns of resource allocation to their offspring, in response to predictive environmental cues (Czesak and Fox 2003 and references therein).

For insects that use discrete resources, such as parasitoids and seed feeders, host size and host quality are major sources of phenotypic variation among host species and may constrain offspring growth influencing the evolution of body size and life history traits (Hardy et al 1992, Allen and Hunt 2001, Mackauer and Chau 2001, Tsai et al. 2001). In species with scramble competition, individuals in populations adapted to large hosts are generally larger than those adapted to small hosts, generating genetic variation in body size among populations adapted to different hosts (Toquenaga and Fuji 1990). Also, because resources are more likely to run out in smaller than in larger hosts, insects mature at smaller size and sooner in small hosts, generating phenotypic variation in body size and development time within populations (Kirk 1991). In our study system, the seed-feeding beetle *Stator limbatus*, beetles in populations adapted to the large-seeded host *Acacia greggii* are about 40% larger than are those adapted to the small-seeded host *Pseudosamanea guachapele*. This difference in body size is likely a consequence of adaptation to large vs. small seeds and is associated with differences in a large suite of growth and life history traits.

The objective of this study was to quantify the relative contribution of environmental (host species), genetic (population) and maternal effects to differences in body size and life history traits between populations of *S. limbatus* developing on seeds of the small-seeded *P. guachapele* and the large-seeded *A. greggii*. Specifically, I asked: (1) What is the magnitude of the genetic differences in body size and life history traits between populations that use hosts of different size? (2) What is the influence of rearing host and oviposition host on body size and fitness related traits? (3) How does maternal rearing host affect the phenotype of their offspring? and (4) Do females exhibit adaptive egg size plasticity in response to the species upon which they were reared or on which they oviposit?

2.2 Materials and Methods

2.2.1 The Beetle

S. limbatus is a seed-feeding beetle (Coleoptera: Chrysomelidae: Bruchinae) distributed from the north of Argentina to the southwestern United States (Johnson and Kingsolver 1976, Johnson et al. 1989). Populations are found mostly in desert or semiarid environments on ≥ 80 host plant species in at least 9 genera throughout its broad distribution. Although *S. limbatus* is considered a generalist because of the large number of hosts it uses, host use varies substantially among localities and most populations use few hosts and are thus specialists relative to the diversity of plant species available to them (Fox et al. 1995, Morse and Farrell 2005a, b).

Females of *S. limbatus* oviposit directly onto the mature seeds of their hosts. After hatching, the larvae burrow into, and develop completely inside the seed. Beetles emerge from seeds as adults and start oviposition 12-48 h later. The complete life cycle takes 28-30 days at 28^{o}C. In another seed beetle, *Callosobruchus maculatus*, pupation represents ~30% of total development, though this is dependent on host species and temperature (Chandrakantha & Mathavan 1986).

The populations used for this study are from Colombia and Arizona (southwestern United States). Each group of populations is in what Morse and Farrell (2005a) show to be different well supported monophyletic clades: the South American clade and the North American clade of *S. limbatus*. Because the two Arizona and the two Colombia populations are more related to each other than to populations from different clades, I expect genetic differences to be larger between populations of different clades than between populations in the same clade.

2.2.2 The Host Plants

I compared populations adapted to the large seeds of *A. greggii* (Arizona, United States) with populations adapted to the much smaller seeds of *P. guachapele* (Cundinamarca and Tolima, Colombia). *A. greggii* (Fabaceae) is a shrub to small tree distributed throughout much of the southwestern United States and northern Mexico (Sargent 1965). It grows in dry areas on gravelly mesas, sides of low canyons and banks of mountain streams. Fruits contain 1 to 5 round, laterally compressed, brown seeds with seed mass typically between 60 to 300 mg. Beetles access seeds by entering the pods through holes made by other insects or through cracks in the pods.

P. guachapele (Fabaceae) is a medium to large tree that grows mostly in pastures and dry areas from Guatemala to Ecuador. The dehiscent fruits have 10-25 small, oval, laterally compressed cream-colored seeds that vary in mass from 18 to 46 mg. Because the pods are dehiscent, beetles have direct access to the seeds once the pods mature.

2.2.3 Field Collection and Colonies Establishment

Beetles were collected on 10-20 August 2002 from *A. greggii* seeds at two localities in Arizona, United States: Wenden 33°49'21’’N; 113°32'27’’W (Yavapai Co.) and Oracle (Pinal Co.) 32°36'39’’N; 110°46'13’’W, henceforth referred to as the “Arizona” populations. Beetles were collected from *P. guachapele* seeds between 28 December 2002 and 10 of January 2003 at two localities in Colombia: Melgar, 4^{o}13’83’’N; 74^{o}37’26’’W (Tolima) and Anapoima 4°31'13’’N; 74°32'22W’’ (Cundinamarca) in Colombia (“Colombia” populations).

Mature fruits were collected from >20 trees at each locality and brought to the lab. Fruits were opened and seeds bearing eggs were placed individually in petri dishes at 28^{o}C. Emerging beetles (> 200) from each population were used to establish laboratory colonies. To remove any environmental effects (Fox et al. 1995) beetles from all populations were maintained in the laboratory at >100 families per generation at 28^{o}C, 15:9 light:dark on seeds of *A. greggii* for two generations (9 weeks) prior to beginning this experiment. Survivorship is high on *A. greggii* seeds for all populations studied here (see Results) such that the rearing of beetles on this host seed imposed at most small amounts of selection on the Colombia populations.

2.2.4 Experimental Design

To distinguish between maternal host vs. rearing host effects I used a two generation rearing design in which half of beetles from each population were raised on seeds of *A. greggii* and the other half were raised on seeds of *P. guachapele*. The emerging offspring from each host were then split into two groups that were mated and had their offspring raised on *A. greggii* (one group) or *P. guachapele* (the other group; Figure 2.1).

The mating procedure for beetles from each population was as follows: 12 hours (h) after emergence from *A. greggii*, three virgin females and two virgin males, all non-siblings, were enclosed in a 60 mm Petri dish with 10 seeds of a single host (either *A. greggii* or *P. guachapele*); these mating groups formed the *Parental Generation.* Beetles were mated in groups of two males and three females because Colombian females rarely lay eggs when kept in pairs (unpublished data). Mating groups were provided with sugar water. Offspring from each of these groups of five beetles was treated in the analysis as a single data point. The dishes were inspected every day until at least 1 egg was laid on each seed, for a total of at least 10 eggs per family. Seeds containing eggs were divided into separate 15 mm Petri dishes (one seed/dish) and allowed to develop at a density of one egg per seed (excess eggs were scraped from the seed). Larvae were raised to adult at 28°C, 15:9 light:dark. These larvae were *Generation 1*.

Generation 1 beetles were sexed and weighed within 12 h of emergence from the seed. Half of these beetles had been raised on *A. greggii* and half on *P. guachapele* seeds. For each group, half of the emerging adults were mated and allowed to oviposit on *P. guachapele*; the rest were mated and allowed to oviposit on *A. greggii* seeds. Larvae were again raised to adult at one individual per seed, 28°C, 15:9 light:dark. These larvae constituted *Generation 2*. Upon emergence these beetles were weighed and sexed.

Sample sizes for each generation were as follows: The *Parental Generation* consisted of 268 groups (families) giving raise to 1543 adult offspring in *Generation 1*. From these *Generation 1* beetles I created 211 groups (families) that produced 1388 offspring in *Generation 2*.

2.2.5 Data Collection

I collected both reproductive data and survival/growth data. Reproductive data were collected for *Generation 1* beetles. These beetles differed in their rearing host (*A. greggii* vs. *P. guachapele*) and in the host upon which they oviposited. I scored adult body mass, age at first reproduction, egg size, and the number of eggs laid during the first 24 h of oviposition (the 24 h after the female's first egg was laid). Survival and growth data were collected on *Generation 2* beetles. These beetles differed in both the host upon which they were raised and the host upon which their mother was raised. I recorded egg hatch, survivorship at different developmental stages (embryo, inside of seeds and total egg–to-adult), egg-to-adult development time (time between when the egg was laid and the adult beetle emerged from the seed), and adult body mass.

All beetles were weighed on an electronic balance (Mettler Toledo AT261 Delta range) to 0.01 mg. I also measured the length of two eggs for each dish using an ocular micrometer; egg length was the average of these two eggs (i.e., one mean egg size per group).

2.2.6 Analysis

For Generation 1 I used ANOVA (Type III sums of squares) to examine clade (country), population (nested within clade), sex, rearing host and oviposition host effects on age at first reproduction, egg size, and the number of eggs laid in the first 24 h of oviposition. I used group means as our lowest level of independence. Analyses in which interactions between variables were non-significant were repeated without the interactions. When the ANOVA yielded significant results, I performed specific post-hoc comparisons between pairs of populations. For Generation 2 I used ANOVA to examine clade, population (nested within clade), sex, rearing host and maternal host effects on body mass and egg-to-adult development time. Survivorship was analyzed using logistic regression.

I used analysis of covariance to determine if differences in egg size among treatments remained significant after controlling for the body size of the females laying those eggs. All statistical tests were done using SAS (SAS Institute, 1985).

2.3 Results

2.3.1 Population Effects (Genetic Effects)

Generation 1 – There were significant differences between the two clades (Colombia vs Arizona) for age at fist reproduction and the number of eggs laid the first 24 h of oviposition. Females from Arizona started to lay eggs sooner after emerging from their host seed than did females from Colombia, regardless of oviposition host (Figure 2.2; Colombia $\overline{X}$ =3.2 ± 0.2 days; Arizona $\overline{X}$ =1.6 ± 0.1 days; $F_{1,193}$=41.1, P<0.0001). Egg size did not differ between clades (Figure 2.3; $F_{1,191}$=0.15, P=0.69) but did differ between populations within clades; Oracle females laid the largest eggs and Wenden females laid the smallest eggs in all treatments. Arizona females also laid more eggs (twice as many) during the first 24 h of oviposition than did Colombia females (Figure 2.4; Colombia $\overline{X}$ =5.9 eggs ± 0.3; Arizona $\overline{X}$ =12.9 ± 1.1; $F_{1,186}$ =43.8, P<0.0001).

Generation 2 – Egg to adult development time and body mass varied among populations. Arizona beetles took longer to develop to adult than did beetles from Colombia (Figure 2.5; Colombia $\overline{X}$ =23.2 ± 0.1 days; Arizona $\overline{X}$ =23.9 ± 0.2days; $F_{1,369}$=4.29, P=0.039). Also, regardless of treatment, beetles from Arizona were substantially larger than beetles from Colombia (Figure 2.6; least squares means after removing treatment effects: Colombia $\overline{X}$ =1.53 ± 0.01 mg; Arizona $\overline{X}$ =2.27 ± 0.04 mg; $F_{3,364}$=387.0, P<0.0001). Males were larger than females in all populations (sex effect $F_{1,364}$=49.2, P<0.0001) as has been shown in other studies with this species. However, the degree of dimorphism differed between clades – Colombian beetles were more sexually dimorphic than Arizona beetles (clade by sex interaction $F_{1,368}$=8.23, P=0.0044). The mean body size difference between sexes in Arizona beetles was 2.4% but for Colombian beetles was 10%.

2.3.2 Rearing host and oviposition host effects

Generation 1 – There was no significant effect of rearing host on the age at first reproduction ($F_{1,193}$=0.02, P=0.9) but females started laying eggs sooner when ovipositing on *A. greggii* (Figure 2.2; laying on *A. greggii* $\overline{X}$ =2.6 ± 0.2 days; laying on *P. guachapele* $\overline{X}$ =2.9 ± 0.2 days; $F_{1,193}$=4.7, P=0.032). There was no significant effect of either rearing or oviposition host on the number of eggs laid in the first 24 h of oviposition (rearing host effect: $F_{1,186}$=2.33, P=0.13; oviposition host effect $F_{1,186}$=0.24, P=0.62)

Females exhibited egg size plasticity in response to their oviposition host. Irrespective of population of origin, females laid larger eggs on seeds of *P. guachapele* than on *A. greggii* (Figure 2.3; average size of eggs laid on *A. greggii* = 0.54 ± 0.004 mm; average size of eggs laid on *P. guachapele* = 0.57 ± 0.007 mm; $F_{1,185}$=37.41, P<0.0001). This difference was still highly statistically-significant after controlling for female body size (i.e., including female body mass as a covariate; host effect on egg size, $F_{1,188}$=42.56, P<0.0001).

Generation 2 – In general, seeds of *A. greggii* were a much better substrate for larval development than were seeds of *P. guachapele*; beetles from all populations experienced higher survivorship and matured sooner and larger when raised on seeds of *A. greggii*.

When performing the logistic regression containing all terms, survivorship at all stages of development was significantly higher when eggs were laid on *A. greggii* (Figure 2.7; survivorship of embryo X^2_1=9.3, P<0.002; egg hatch X^2_1=21.2, P<0.0001; survivorship of larvae and pupae inside the seed X^2_1=21.1, P<0.0001; survivorship from egg to adult; X^2_1= 8.5, P<0.004). However, survivorship was fairly high at all stages of development and thus effect sizes were small (Figure 2.7). Also, the effect of rearing host differed between maternal host treatments (see *Maternal rearing host effects* section below).

Egg to adult development time was longer inside of *P. guachapele* seeds than inside *A. greggii* seeds (Figure 2.5; 2.1 d longer in males and 2.2 d longer in females; $F_{1,369}=103.6$, $P<0.0001$). This pattern was still significant after controlling for maternal egg size (host effect after controlling for maternal egg size, $F_{1,375}=103.15$, $P<0.0001$) and offspring body mass (host effect after controlling for body mass, $F_{1,378}=38.56$, $P<0.0001$), though beetles that matured larger also matured sooner (slope = -0.17 d/mg; $P=0.0006$).

Despite taking longer to reach maturity, beetles raised on *P. guachapele* were smaller than beetles raised on *A. greggii*, regardless of their native or maternal host (Figure 2.6; average size of beetles emerging from *A. greggii* =1.94 ± 0.37 mg; average size of beetles emerging from *P. guachapele* = 1.61 ± 0.02 mg; $F_{1,364}=367.8$, $P<0.0001$). Although beetles from all populations were larger when raised on *A. greggii*, the effect of rearing host differed between beetles from the two clades and differed between the sexes (clade by rearing host by sex interaction $F_{1,368}=8.23$, $P=0.004$). Beetles from Arizona, which are much larger than beetles from Colombia, were more negatively impacted by rearing on *P. guachapele* seeds than were the smaller-bodied Colombian beetles; Arizona beetles were > 20% smaller when raised on *P. guachapele* (relative to being reared on *A. greggii;* females were 29.5% smaller and males were 24.5% smaller), whereas Colombian beetles were only 11.0% (females) and 16.5% (males) smaller when raised on *P. guachapele*.

2.3.3 Maternal rearing host effect

A significant maternal host x clade interaction was found for egg hatch ($X^2_1=4.72$, $P=0.02$), survivorship inside of the seed ($X^2_1= 8.12$, $P=0.004$) and egg to adult survivorship ($X^2_1=8.44$, $P=0.004$). However, though statistically significant, the patterns are unclear (Figure 2.7).

More evident is the result that beetles whose mothers were reared on *P. guachapele* emerged about one day sooner than beetles whose maternal host was *A. greggii* regardless of the host on which progeny were reared (Figure 2.5; maternal host *P. guachapele,* $\overline{X}$ =23.0 ± 0.1 days, maternal host *A. greggii,* $\overline{X}$ =23.8 ± 0.2 days; $F_{1,369}$=12.6, *P*=0.0004). This effect of maternal host was still highly significant after controlling for egg size ($F_{1,375}$=15.4, *P*=0.0001) and for the mass of offspring ($F_{1,378}$=18.7, *P*<0.0001). Despite maturing sooner, offspring from mothers reared on *P. guachapele* were not smaller ($F_{1,368}$=0.49, *P*=0.48) indicating that maternal host affected development rate and not just development time. This result is contrary to the effect of rearing host on development time; beetles from all populations matured sooner (Figure 2.5) and at much larger body size (Figure 6) when raised on *A. greggii*.

2.4 Discussion

2.4.1 Population Differences and Plastic Responses to Host Species

Even though populations from the two clades (Colombia and Arizona) exhibited significant genetically-based differences in body size and life history traits, all populations of *S. limbatus* were phenotypically plastic in response to host species; they developed faster and matured at a larger size inside *A. greggii* seeds than inside *P. guachapele* seeds. This plasticity may be in response to seed size or seed quality – *A. greggii* are substantially larger seeds, but may also be a better nutritional source. In agreement with the usual expectations for scramble-competing species (Hardy et al. 1992, Tsai et al. 2001) beetles matured at larger size when developing on large seeds. However, contrary to the typical host size effects, beetles also matured sooner on the large-seeded species (and thus had a higher growth rate). This is consistent with results from studies showing that development time decreases and adult mass increases when insects develop on high quality hosts (Lindroth et al. 1991; Stockhoff 1993). I thus believe that many of the host effects observed here are due to nutritional differences between the species rather than just seed size effects.

These data also suggest that large-bodied beetles (e.g., from Arizona) suffer greater fitness costs than do small beetles when raised on small seeds – although beetles from all populations matured smaller when raised on the small seeds of *P. guachapele*, beetles from Arizona (which are larger) were affected most by host species. This result does not directly demonstrate selection on body size, but is suggestive. The seed-beetle for which the effects of host size on body size are best studied is *Callosobruchus maculatus* in which intense larval competition inside small seeds drives the evolution of contest competition favoring large larvae and leading to the evolution of large body size (Messina 1991 a,b, 2004, Toquenaga 1993). In contrast, populations adapted to larger-seeded hosts evolve scramble competition with larvae feeding at the periphery of the seeds where the probability of encountering other larvae decreases. The absence of contest competition allows the evolution of small adults (Credland et al. 1986) possibly because maturing sooner (and thus smaller) reduces the probability of encountering potential competitors and reduces generation time. At the moment, there is no evidence that contest competition evolves in *S. limbatus*. Larval survival is high even at high larval density on small *A. greggii* seeds.

2.4.2 Maternal host effects

Maternal effects are widespread among all types of organisms (Gil et al. 1999, McIntyre and Gooding 2000a, Agrawal 2002, Reinhold 2002). In insects, they influence a large number of traits including larval survival, development time, wing morph, and sex ratio. Maternal effects also provide a mechanism by which organisms can deal with variable environments (Fox and Mousseau 1998). In generalist herbivorous insects, different host plants represent different sets of chemical and physical conditions with which offspring must cope. Female rearing environments, and their oviposition experiences, provide females information on which hosts their offspring will encounter. Females thus have the opportunity to modify traits such as egg size and composition (e.g., maternally-derived proteins and mRNAs) to prepare offspring for the expected host species. Although many studies have now shown effects of maternal diet on offspring growth and development (reviews in Fox et al. 1995, Spitzer 2004) few have demonstrated that maternal effects based on resource use are adaptive (Spitzer 2004). Those examples of adaptive resource-based maternal effects are largely cases in which females respond to host species or host quality to regulate offspring flight morphs (review in Fox & Mousseau 1998) or for which females manipulate egg size in response to oviposition substrate (see *Egg size plasticity* section, below) or in response to food stress (e.g., many cladocerans; discussed in Fox and Czesak 2000).

In this experiment *S. limbatus* offspring matured sooner (shorter egg-to-adult development time), regardless of rearing host, when the maternal rearing host was *P. guachapele.* This result is contrary to the effect of rearing host on development time; beetles from all populations matured sooner when raised on *A. greggii*. However, this result is similar to a maternal effect found for *S. limbatus* by Fox et al. (1995) in which offspring matured sooner when mothers had been reared on *Parkinsonia florida*, rather than *A. greggii*, regardless of offspring rearing host. That maternal effect was also contrary to the direct effect of rearing host on offspring – offspring reared on *P. florida* matured later than offspring reared on *A. greggii*. Fox et al. (1995) also found that maternal rearing host affected offspring body size (offspring were larger when their mothers were raised on *P. florida*), but no such effect was found in this current study. Neither Fox et al. (1995) nor this current study found any evidence that offspring have higher fitness (higher survivorship, reduced development time or larger body size) when raised on the same host as their mother (i.e., no significant maternal host x offspring host interactions). These data thus indicate that maternal rearing host affects offspring through some as yet unclear mechanism, but I have no evidence that *S. limbatus* mothers prepared their offspring for the specific host that the mothers had encountered (no evidence of adaptive “conditioning” or “acclimatization”, following the terminology of Via 1991 and Spitzer 2004, respectively). However, the observed maternal effect may be adaptive – though females do not prepare their offspring for a specific host, they may respond to the poor quality of their rearing substrate by changing their allocation to eggs, so that their offspring are better prepared to tolerate food stress or a lower quality host. This type of maternal effect has been observed in many cladocerans (e.g., food-stressed females lay larger and more energy rich eggs; references in Glazier 1992).

However, the adaptive significance, if any, of the maternal host effect observed in *S. limbatus* needs to be examined further.

The mechanism for the maternal rearing host effect in *S. limbatus* is not known. Previous studies have shown that maternal effects on offspring development time are often due, at least in part, to effects on egg size (Fox 1997a, Fox 1997b, Fox et al. 1999). Despite the regular result that variation in egg size mediates variation in development time, the maternal rearing host effect on development time observed in the current study is apparently not due to changes in egg size; egg size was not affected by maternal rearing host and the maternal host effect on development time was still statistically highly significant after including egg size as a covariate in the statistical model. The observed maternal rearing host effect is thus more likely due to changes in egg composition, such as egg energy reserves, maternally produced proteins (such as regulatory proteins or enzymes), or maternal mRNAs. Unfortunately, how maternal effects influence the composition of eggs is poorly studied in arthropods other than *Drosophila* (Rushlow et al. 1987, Girton and Jeon 1994). For herbivores it is known that egg energy reserves change with maternal age (McIntyre and Gooding 2000a) and female nutritional status (Murphy et al. 1983, Wallin et al. 1992, Fox and Dingle 1994), and that compounds sequestered by parents during development can be passed to offspring (Hartmann et al. 2004, Sime et al. 2000), but little else is known.

2.4.3 Egg size and egg size plasticity

Despite their much smaller body size, females from Colombia laid eggs similar in size to those laid by the much larger bodied Arizona beetles. Arizona *S. limbatus* are largely capital breeders – they use primarily larval-acquired resources for producing eggs such that producing large eggs comes at a substantial fecundity cost to females (adult females will feed, and feeding does prolong their life, but it has very little effect on total fecundity). Though I did not quantify lifetime fecundity in this study, our data do show that fecundity in the first 24 h of oviposition is much lower in Colombian beetles than in Arizona beetles, as expected from their large egg size relative to their body size.

Also, unpublished data (A. Amarillo) indicate that lifetime fecundity in the lab is very low for Colombian beetles and females do not lay eggs unless food is provided. Because selection for high fecundity is strong, the selection for high fecundity may be balanced by very strong selection favoring large eggs in Colombia beetles and, unlike Arizona beetles, Colombian beetles may use (and even require) adult food sources to produce eggs (i.e., they are income breeders). Such variation in allocation strategies (capital v. income breeding) within a species provides an exciting opportunity to study the evolution of allocation strategies.

Within the Coleoptera and Lepidoptera there are a number of species that exhibit egg size plasticity in response to host species and/or quality (Leather and Burnand 1987, Nylin and Gotthard 1998, Awmack and Leather 2002, Ekbom and Popov 2004, Takakura 2004). Plastic responses to host quality, like the responses I observed in *S. limbatus,* are a strategy that allows organisms to cope with variation among hosts. Previous studies with *S. limbatus* have shown that females adjust the size of eggs they lay in response to the oviposition host species, but not in response to variation in the size of seeds within species (Fox et al. 1997, Savalli and Fox 2002). Specifically, populations of *S. limbatus* from Arizona and Texas (USA) lay larger eggs on seeds of *Parkinsonia florida* (which produces seeds very resistant to larval penetration) than on seeds of either *A. greggii* or *P. microphylla* (which produce non-resistant seeds). This plasticity appears to be adaptive. Offspring from larger eggs have much higher survival during penetration of *P. florida* seed coats (thus selection favors large eggs on this host) but females laying larger eggs have substantially reduced fecundity relative to females laying smaller eggs (thus, selection favors small eggs on *A. greggii* and *P. microphyllum*, on which larval survival is high for small eggs).

In this current study I found that females from all populations laid larger eggs when ovipositing on *P. guachapele* than when ovipositing on *A. greggii* (note that I found no effect of maternal *rearing* host on egg size, but did find a large effect of maternal *oviposition* host on egg size). This is the first time plasticity in *S. limbatus* has been demonstrated to increase egg size in response to a host species other than *P.*

florida. In contrast to the egg size plasticity exhibited by Arizona beetles in response to *P. florida*, the host effect on egg size observed here does not appear to be due to selection to overcome seed coat defenses. Larval mortality on *P. guachapele* was not affected by egg size. The larger eggs laid on seeds of *P. guachapele* may be an adaptive strategy that helps larvae compensate for the low quality and/or size of *P. guachapele* seeds; females may lay larger eggs either (a) as a mechanism to promote development on a poor quality nutritional source or (b) to prepare larvae for the small size of their host seed and the larval competition they are likely to experience. These hypotheses have yet to be tested.

Stator is a genus of beetles that mainly use seeds of legumes. Most species are specialists in that they use just a couple of species as hosts. In contrast *S. limbatus* has colonized ~ 80 legume species across all three legume families. They thus must cope with wide variation in seed quality, chemistry and size. Specialization on *Acacia* appears to be the ancestral trait in the genus *Stator* and the generalist diet of *S. limbatus* appears to be derived from *Acacia* specialized ancestors (Morse and Farrel 2005a). It is likely that the phylogenetic constraint on diet evolution (feeding on *Acacia*) was overcome in *S. limbatus* by the evolution of egg size plasticity, allowing the species to colonize a wide diversity of host species and become a relative generalist. The Colombian and Arizona populations both responded to *P. guachapele* by increasing egg size (relative to the size of eggs laid on *A. greggii*). Both populations also respond to *P. florida* by laying large eggs (Chapter three). That Colombian and Arizona populations are located on very divergent clades (Morse and Farrell 2005a) supports the hypothesis that egg size plasticity is ancestral within *S. limbatus*; that egg size plasticity evolved before the divergence between clades is more parsimonious than the alternative hypothesis that egg size plasticity evolved separately in each clade. Recent studies of *S. limbatus* colonization of non-native (ornamental or invasive plants) species in the southwestern United States support this hypothesis – the survival of offspring on novel hosts following colonization is influenced by female experiences pre-colonization and the effects of these experiences on the size and composition of eggs laid by females (Fox 2006a).I propose that egg size plasticity is an adaptive trait that has played an important role in diet expansion and diversification in *S. limbatus* and may be the feature of this beetle's life history that allowed it to evolve a generalist life style.

In conclusion, I have demonstrated that populations of *S. limbatus* that use different hosts have diverged in body size and life history traits. However, all *S. limbatus* populations exhibited substantial host-associated phenotypic plasticity. This plasticity, both by offspring (e.g., development time and body size) and their mothers (egg size plasticity, which affects offspring as a maternal effect) likely buffers these beetles from high mortality or low fitness that they would otherwise experience when encountering novel hosts, and thus likely facilitates colonization of novel hosts. However, phenotypic plasticity also buffers organisms from selection post-colonization reducing the rate at which populations adapt to novel hosts (Strauss et al. 2006). In addition, plasticity in responses to novel environments (e.g., host species) may be asymmetrical with some populations (e.g., large-bodied Arizona populations of *S. limbatus*) experiencing greater fitness costs than others (e.g., small-bodied Colombia populations) when exposed to lower quality (e.g., smaller-seeded) hosts. Disentangling the historical role of plasticity in mediating the colonization of new environments, and subsequent adaptation to those environments, requires consideration of the phylogenetic history of the species and populations being studied.

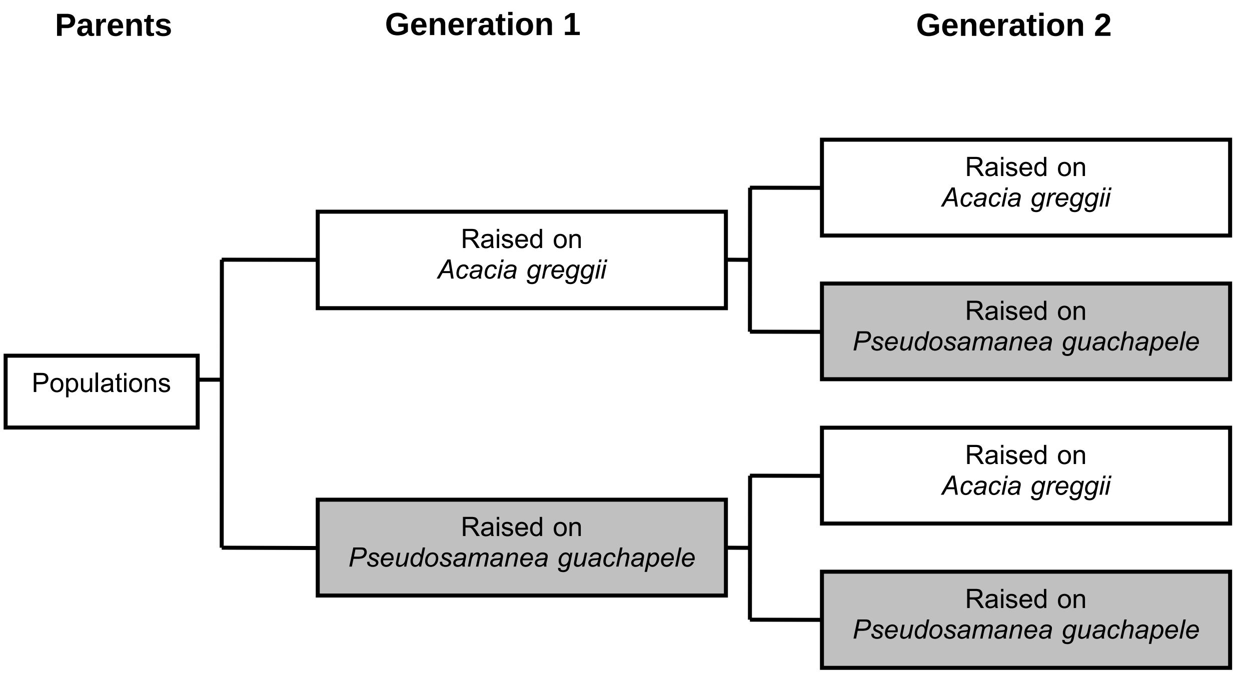

Figure 2.1: Three generation rearing design used to evaluate maternal, oviposition and rearing host effects in four populations of the seed-feeding beetle *S. limbatus*. Beetles were reared on either *Acacia greggii*, a large-seeded host or *Pseudosamanea guachapele*, a small-seeded host.

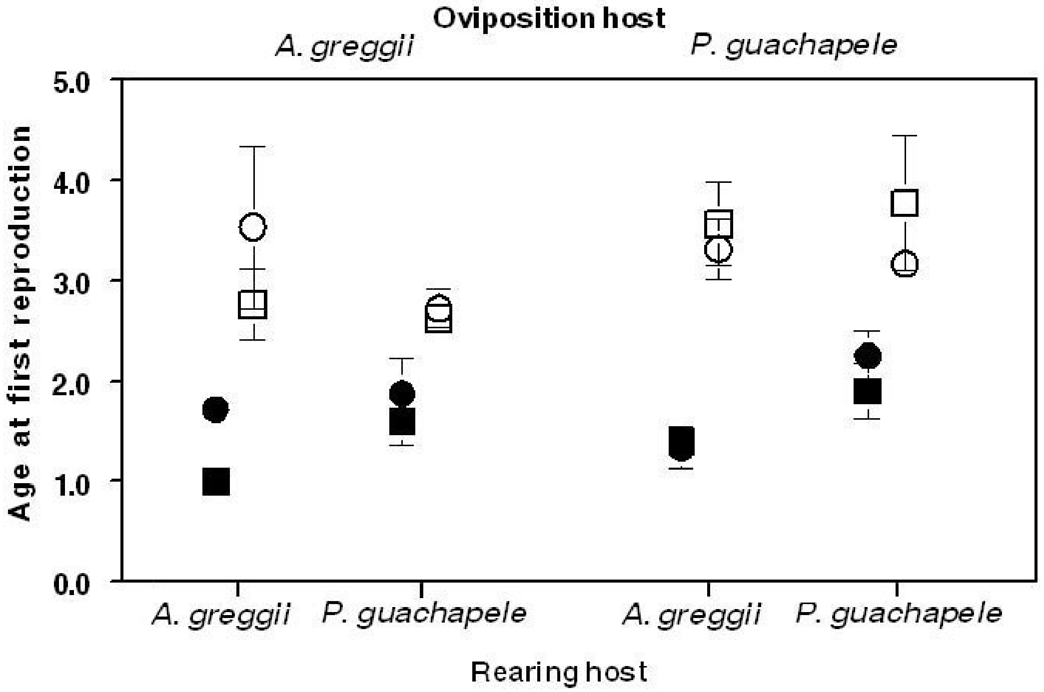

Figure 2.2: Effect of oviposition host and rearing host on age at first reproduction of females from four populations of *S. limbatus*. Solid symbols indicate populations from Arizona, USA (Oracle (■), Wenden (●)). Open symbols indicate populations from Colombia (Anapoima (□), Melgar (○)). Standard error bars for some points are in some cases smaller than the symbols. The means presented are averages of group means for each treatment-population combination.

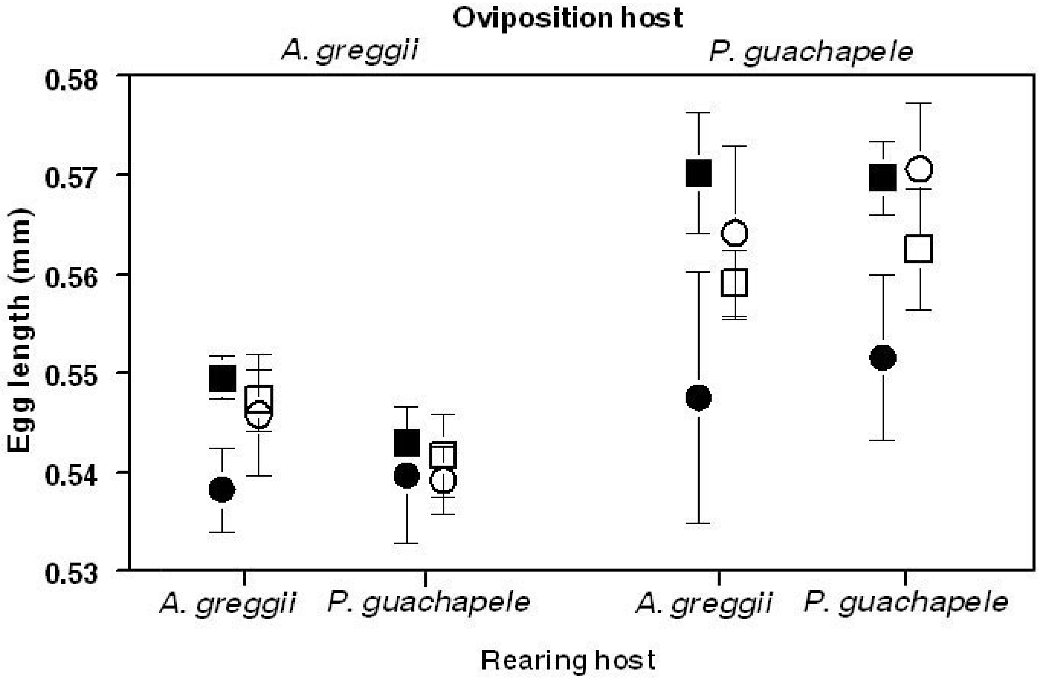

Figure 2.3: Effect of oviposition host and rearing host on the size of eggs laid by females of four populations of *S. limbatus*. Solid symbols indicate populations from Arizona, USA (Oracle (■), Wenden (●)). Open symbols indicate populations from Colombia (Anapoima (□), Melgar (○)). Standard error bars for some points are smaller than the symbols.

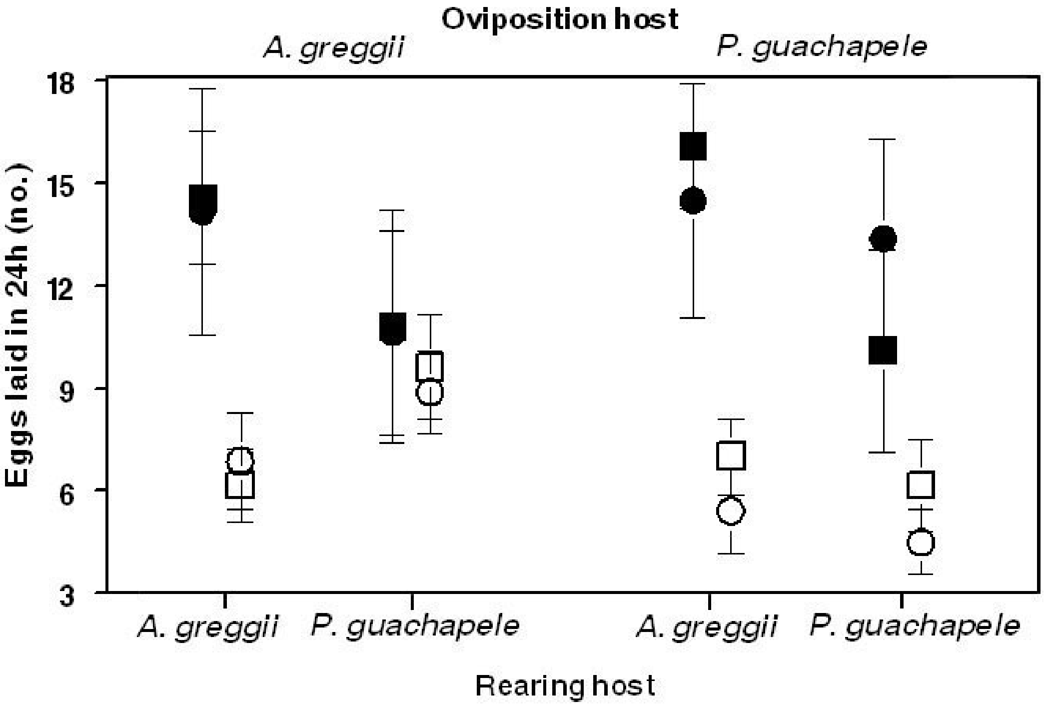

Figure 2.4: Effect of oviposition host and rearing host on the number of eggs laid during the first 24 h of oviposition for females from four populations of *S. limbatus*. Solid symbols indicate populations from Arizona, USA (Oracle (■), Wenden (●)). Open symbols indicate populations from Colombia (Anapoima (□), Melgar (○)). Standard error bars for some points are in some cases smaller than the symbols.

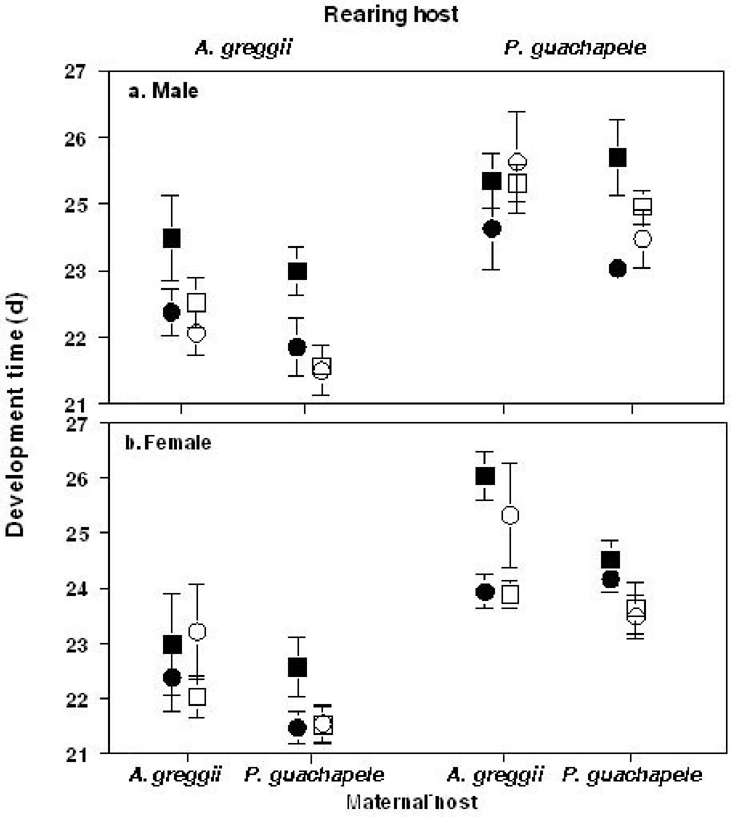

Figure 2.5: Effect of maternal host and rearing host on egg-to-adult development time of a. Male and b. Female beetles from four populations of *S. limbatus*. Solid symbols indicate populations from Arizona, USA (Oracle (■), Wenden (●)). Open symbols indicate populations from Colombia (Anapoima (□), Melgar (○)). Standard error bars for some points are smaller than the symbols.

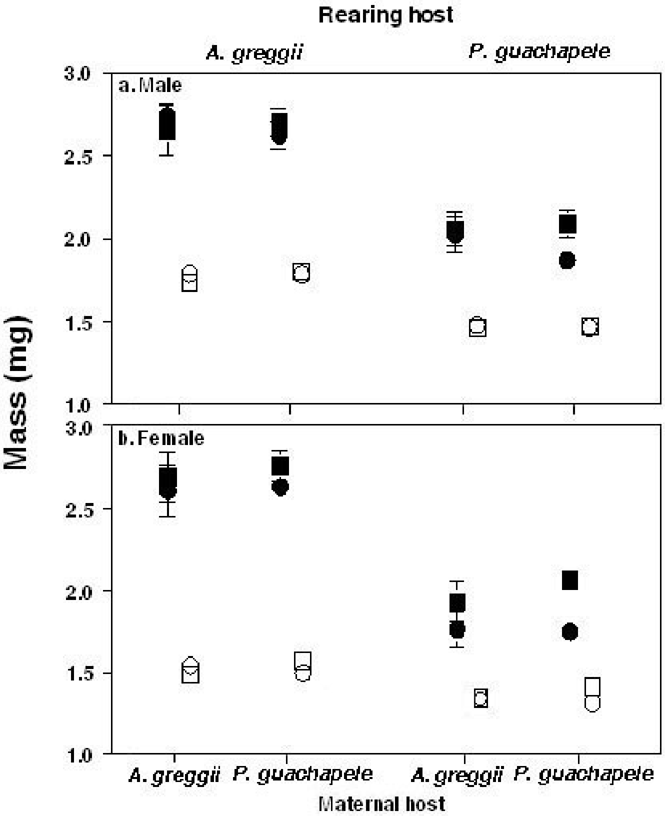

Figure 2.6: Effect of maternal host and rearing host on body mass of beetles from four populations of *S. limbatus*. A, Males; B, Females. Solid symbols indicate populations from Arizona, USA (Oracle (■), Wenden (●)). Open symbols indicate populations from Colombia (Anapoima (□), Melgar (○)). Standard error bars for some points are in some cases smaller than the symbols.

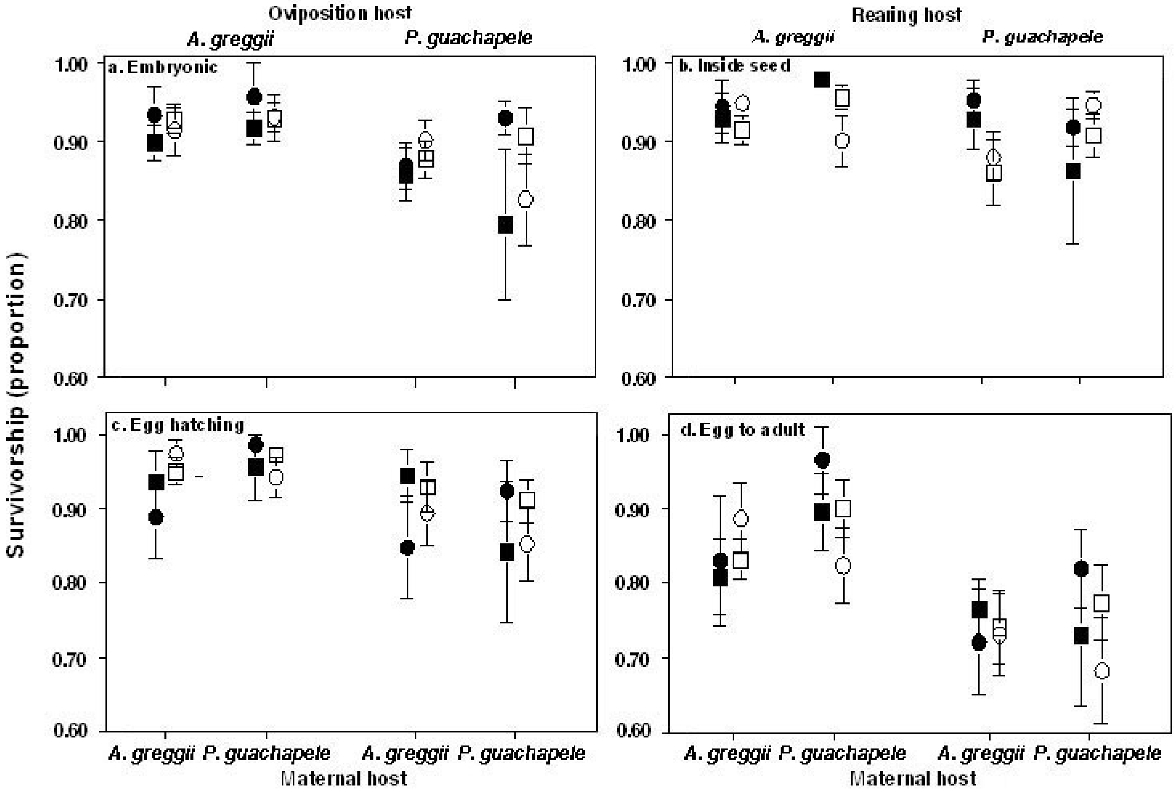

Figure 2.7: Effect of maternal host and oviposition-rearing host on survivorship at different developmental stages (A-C) and total egg-to-adult survivorship (D) for four populations of *S. limbatus*. Solid symbols indicate populations from Arizona, USA (Oracle (■), Wenden (●)). Open symbols indicate populations from Colombia (Anapoima (□), Melgar (○)). Standard error bars for some points are smaller than the symbols.

Chapter 3: Host Discrimination and oviposition behavior in *Stator limbatus*

3.1 Introduction

In parasitic insects that use discrete hosts, such as other insects or seeds, and for which larvae are unable to move among hosts, variation in host size and quality can have direct and substantial impact on larval development (Mangel 1992). Because larger hosts provide more nutrients or a greater quantity of food for the offspring (Yang et al 2006), progeny developing in larger hosts usually have lower mortality, greater fecundity and reach larger body size, which contributes to increased fitness (Godfray et al. 1991, Heimpel et al. 1996,). In some cases, large hosts also may confer greater space for parasitoid avoidance (Freese 1995).

Oviposition decisions made by females may have large effects on both maternal and offspring fitness and in consequence, is expected that females would exhibit preference for ovipositing on larger and better quality hosts by discriminating the size and the number of eggs already laid on that host (Godfray 1987, Godfray et al. 1991, Broudeur and Boivin 2004). This prediction has been well supported by a number of studies that show that females of many species prefer to oviposit on non-parasitized rather than parasitized hosts, and in larger rather than smaller hosts (Fox and Mousseau 1995, Godfray et al. 1991, Awmack and Leather 2002). For example, females of *Callosobruchus maculatus*, a seed beetle for which oviposition host discrimination has been well studied, generally distribute their eggs uniformly among the seeds available (Messina 1989), deposit more eggs on larger hosts and are more likely to super-parasitize larger than smaller hosts (Cope and Fox 2003), though female oviposition behaviors and preferences are genetically variable, both within and between populations.

Stator limbatus is a Bruchinae beetle (Coleoptera: Chrysomelidae: Bruchinae) in which larval development occurs completely inside a single seed and thus female oviposition decisions will have large effects on offspring fitness. This species feeds on seeds of about 80 host plants throughout its broad geographic range (Johnson and

Kingsolver 1976), but individual populations usually have just a few hosts available. In addition, host species vary in seed size and quality among and within localities. Adaptation to the specific hosts available at each locality have influenced the evolution of beetle body size, age at first reproduction and development time among others (Chapter two). Females can also manipulate egg size and egg content in response to host species (Fox et al. 1999).

In this study I examine host discrimination and oviposition behavior of female *S. limbatus* from populations locally adapted to different hosts (hosts of differing seed size and quality). I performed four experiments in which I addressed the following questions:

(1) When encountering seeds that vary in the density of conspecific eggs, how does the number of eggs present on a seed, and their developmental stage (hatched versus unhatched) affect female fecundity and egg dispersion? For insects that undergo development inside discrete resources such as parasitoids and seed feeders, female discrimination at oviposition affects offspring fitness (Yang et al. 2006). Studies with some insects have shown that females avoid host plants bearing con-specific eggs, leading to non random distribution of eggs among the available hosts (Messina and Dickinson 1993). For example, *Callosobruchus maculatus* females tend to distribute eggs uniformly among seeds (Messina and Mitchell 1989) and females prefer to oviposit on seeds with a lower than average number of eggs (Messina and Renwick 1985), though the degree of egg avoidance varies substantially among populations. This behavior leads to lower competition among siblings and in consequence generates higher survivorship, increasing female fitness (Wilson 1988). Because *S. limbatus* is a seed feeder with scramble competition, it is expected that females will distribute eggs non-randomly among seeds to minimize larval competition experienced by their offspring. Thus, the objective of this experiment was to determine the effects of seeds bearing different number of eggs, and at two different developmental stages, on female's fecundity and egg dispersion.

(2) Do females prefer to oviposit on larger seeds? Previous studies with species of herbivorous and parasitoid insects show that larger hosts provide more nutrients to the developing larvae (Godfray et al. 1991 and references there in) and reduce the deleterious effects of competition among siblings. Compared with insects developing in small hosts, insects developing in larger hosts experience longer development time and increase body size, because they can make use of the additional resources to attain larger size (Mackauer and Chau 2001). These responses are associated to organisms with scramble competition in which immature share the same discrete patch. Studies done with *S. beali*, a specialist seed beetle on large seeds of Texas ebony, (*Chloroleucon ebano* Berlandier) show that females prefer to oviposit on large seeds when presented with both a large and a small seed (Fox and Mousseau 1995). The *S. limbatus* populations examined here all exhibit scramble competition. Thus, I expect ovipositing females to prefer larger over smaller hosts. However, the effect of seed size on female preference could be also influenced by female body size. Given that female body size is positively correlated with fecundity, clutch size and offspring size (Fox 1994, Visser 1994, Allen and Hunt 2001) small females would lay fewer eggs and smaller clutches than large females, and in consequence it would be expected than smaller hosts would not be as restrictive for offspring development of small beetles. Thus, the objective of this experiment was to establish female's oviposition preference for large and small seeds when exposed to mixed or non choice treatments differing in seed size.

(3) How is oviposition preference of females affected by variation in size versus quality of seeds? When adaptation to a local host occurs, populations differing in host availability generally evolve preferences for their native host. This can reduce their willingness to accept alternate hosts if there is a trade-off between adaptation to the local host and the ability to use novel hosts (Mopper et al. 2000). However, preference for a better quality host, irrespective of the native host, could also drive females to prefer lay eggs on non native hosts. The objective of this experiment was to determine whether populations of *S. limbatus* adapted to host seeds differing in size and quality exhibit oviposition preference for their native host regardless of the size and quality of

the alternative host. In addition, because populations tested in this experiment differ in body mass as a result of adaptation to seed hosts (Chapter 2), I tested whether body mass affected preference for seeds.

(4) Do females adjust egg size similarly in response to both native and non-native resistant or low quality hosts? Studies examining the fitness consequences of egg size on progeny show that progeny hatching from larger eggs had higher fitness or better performance than progeny hatching from small eggs (Fox and Czesak 2000). Variation in egg size within populations had been demonstrated in response to several environmental factors such as maternal diet, rearing and oviposition temperature, maternal density, seasonal variation and oviposition host (Fox and Czesak 2000 and references there in). Egg size plasticity in *S. limbatus* has been demonstrated for populations in Arizona, which use *A. greggii* and *Parkinsonia florida* as major hosts. When ovipositing on *Pa. florida,* females lay larger eggs and larvae have higher probability of burrowing into the seed after overcoming the toxic seed coat (Fox et al 1997). *Pseudosamenea guachapele* seeds are smaller and development of beetles takes longer than development in *A. greggii* seeds (Chapter two), suggesting that *Ps. guachapele* seeds are a low quality nutritional resource for larvae development. In addition, variation in egg size among populations within species is commonly associated to latitudinal and altitudinal clines, with smaller eggs produced at lower latitudes and altitudes, although for many arthropods variation in egg size is more associated with changes in host plants (Fox and Czesak 2000), a very determinant factor when herbivorous live in a variable environment with habitat heterogeneity across time or space, or when populations use each a different host, having an optimal phenotype (Futuyma 2001). The seeds used in this experiment are *Ps. guachapele*, *Pa. florida* and *A. greggii*. *Ps. guachapele* is a host that restricts survivorship given the small size and poor nutritional quality of its seeds, and *Pa. florida* a host that limits survivorship given the presence of secondary compounds in the seed coat. *A. greggii* is the seed in which beetles have higher survivorship. Thus, the objective of this experiment was to compare the ability of females from populations adapted to different

host plants to exhibit egg size plasticity when exposed to seeds of *Ps. guachapele*, *Pa. florida and A. greggii*.

3.2 Methods and Results

3.2.1 Beetle species and source populations

Stator limbatus is a seed-feeding beetle (Coleoptera: Chrysomelidae: Bruchinae) with a broad distribution in the Americas, ranging from the southwestern United States in North America to the northwest of Argentina in South America (Johnson and Kingsolver 1976). Larval development occurs inside of seeds of ~80 different plant species, but populations have only a few hosts available at most localities. Females lay eggs directly onto the surface of seeds and first instar larvae burrow into the seed underneath the egg. Larval development takes place inside a single seed and larvae can not move among seeds.

The populations used in these experiments were collected in Arizona and Texas in the United States and in Cundinamarca and Tolima in Colombia. Populations from the United States were collected from *A. greggii* in Oracle, (Pinal Co.; 32°36'39’’N; 110°46'13’’W on August 2002), from *Pa. florida* in Phoenix (Arizona, August 2001) and from *A. berlandieri* in Del Rio (Texas; 29°28'31’’N; 100°59'21’’W, August of 2003). Populations from Colombia were collected from *Ps. guachapele* in Melgar (Tolima; 4°13'45’’N; 74°13'91’’W) and Anapoima (Cundinamarca; , 4°31'13’’N; 74°32'22’’W) from December (2002) to January (2003). Beetles from the Colombia populations do not have access in the field to *Pa. florida* nor to *A. greggii*, and beetles from the United States populations do not have seeds of *Ps. guachapele* available.

I collected seeds from >20 trees at each locality and stored them on hermetic bags to be transported to the laboratory. Seeds bearing eggs were enclosed in Petri dishes at 28^{o}C. Emerging beetles were used to establish laboratory colonies; >200 beetles emerged for all four populations. Colonies were maintained at ≥100 families each generation. Because Colombian females do not lay eggs when enclosed with a single male, families were created by mating two females with one male. After two generations of laboratory rearing on their natural hosts, beetles from all colonies were transferred onto *A. greggii* seeds for one generation (to remove host-associated environmental effects). Beetles from all populations have very low mortality on seeds of *A. greggii* (Chapter 2).

3.2.2 Host species

I used seeds of three species of *S. limbatus* hosts for these experiments. Seeds of *A. greggii*, a host of the Arizona populations, were used for the experiments examining beetle preferences for large versus small seeds. *A. greggii* (Fabaceae) is a small to medium size tree that grows in semiarid areas throughout much of the southwestern United States and northern Mexico (Sargent 1965). Seed pods have between 1 and 5 brown seeds with seed mass typically between 60 to 300 mg. Seeds of *Pa. florida*, also a common host used by Arizona populations, were used for the experiment on egg size plasticity. Seeds of this species are similar in size to seeds of *A. greggii*, but have a toxic substance in the seed coat that causes high mortality of larvae burrowing into the seed (Fox et al. 1997). Seeds of *Ps. guachapele,* the host of the Colombia populations, were used in the experiments examining female oviposition preferences and egg size plasticity. This species is a medium to large tree that grows mostly in pastures and dry areas from Guatemala to Ecuador. The dehiscent seed pods have 10-25 small seeds that vary in mass from 18 to 46 mg. Because pods are dehiscent, beetles have direct access to the seeds once mature.

3.2.3 Criteria to measure seed size preference and seed host preference

Preference of females for seeds of a specific size or species was quantified in one of three ways, depending on the experiment: (a) the total number of eggs laid on seeds during a fixed period of time or until at least ten eggs were laid, (b) the proportion of females that laid at least one egg on each seed type, and (c) the amount of time that females delayed oviposition on a seed type. In experiment two, females were exposed to a mixed seed size treatment (one large – one small *A. greggii* seed) and to two same seed size treatments (two large or two small *A. greggii* seeds). In experiment three females were exposed to three different seed size/host treatments (large or small *A. greggii* with *Ps. guachapele* seeds).

Information about preference was obtained by determining number of eggs laid and the time to start ovipositing on each seed size/type. It is expected that females would lay more eggs and start to lay eggs sooner on the most preferred host. Information about acceptability was obtained by determining the proportion of females that laid at least one egg on each seed size/type. It is expected that females that lay at least one egg on the seed already accept the host even if this is not the most preferred. I also measured acceptability in experiment two in the non choice treatments. Details about the procedure for each experiment are given below in the sections explaining each individual experiment.

3.2.4 Experiment 1: Effects of the number of eggs present on a seed, and their developmental stage (hatched versus unhatched) on female fecundity and egg dispersion.

3.2.4.1 Methods

I tested the hypothesis that females are deterred from laying eggs on seeds bearing conspecific eggs relative to seeds without eggs, and that eggs laid on seeds already bearing eggs are distributed less uniformly than eggs laid on clean seeds. For this experiment I used beetles from the Oracle and Phoenix populations (both from Arizona) that use both *A. greggii* and *Pa. florida* seeds in nature.

520 pairs of beetles were confined in 35 mm Petri dishes (one pair per dish) with seeds bearing either zero, one, two, or three eggs per seed. In half of the replicates eggs on these seeds were hatched and in the other half eggs were unhatched.

To obtain seeds with hatched and unhatched eggs at the three required egg densities, 15 groups of 200, 24h old virgin beetles were enclosed in boxes containing either 300 or 500 clean (egg-free) seeds of *A. greggii*. Seeds were previously sieved to standardize size to a diameter of 10-12mm. Half of these eggs were allowed to hatch (after which they were frozen), and the other half were frozen two days after being laid.

To determine how the presence of eggs, egg density, and the developmental stage of eggs (hatched versus unhatched) affects female egg laying, pairs of virgin beetles, 24 h post-emergence, were enclosed for 24 hours in Petri dishes without seeds and allowed to mate. 24 h later each mated pair was enclosed in a Petri dish containing 10 seeds bearing 0, 1, 2, 3, or 4 hatched or 0, 1, 2, 3, or 4 unhatched eggs (only one egg density or egg treatment per dish). The pair was allowed to oviposit for 24 h, then was transferred to a new Petri dish containing 20 seeds with the same egg density per seed and allowed to oviposit until death. The total number of eggs laid by each female was counted. An analysis of variance was used to test for effects of conspecific egg density and egg developmental stage (hatched versus unhatched) on the number of eggs laid by females. Because there was no effect of replicate (population) on mean fecundity, ($F_{1,283}$=2.86, P=0.09) analyses and graphs show pooled results.

To determine how the presence of conspecific eggs affects the dispersion of new eggs laid by females, I recorded the distribution of eggs among seeds during the first 24 h. period of oviposition and estimated the degree of uniformity of eggs among seeds following Messina and Mitchell (1989). This uniformity index is based on the number of “mistakes” committed by a female in distributing her eggs, with a “mistake” defined as the number of eggs that need to be relocated among seeds to obtain the most uniform distribution possible. This index is given by the algorithm U = (E – O)/E, where E is the expected number of mistakes and O the observed number of mistakes. The index usually ranges between 0 and 1, where 0 represents a random distribution and 1 represents a uniform distribution. U will be less than 0 if a female clumps her eggs. I tested for treatment effects using Analysis of Variance. All statistical analyses were performed using SAS (SAS Institute 1985).

3.2.4.2 Results

Females laid more eggs on clean seeds than on seeds bearing eggs, and female fecundity declined as the number of conspecific eggs increased (Figure 3.1; $F_{3,344}$ =4.7; P=0.0031). However, there was no significant effect of the developmental stage of eggs (hatched vs. unhatched) on female fecundity ($F_{1,344}$=1.24; P=0.26).

Irrespective of the type of eggs ($F_{3,255}$=2.50; P=0.11) egg dispersion decreased with increasing number of eggs previously laid on the seed (Figure 3.2; $F_{3,255}$ = 2.77; P = 0.04). That is, as the number of eggs previously laid in the seeds increased, females distribute their eggs less uniformly.

3.2.5 Experiment 2: Preference of females for large versus small seeds

3.2.5.1 Methods

I tested two hypotheses: (a) Females start ovipositing earlier and lay more eggs when enclosed with large seeds than when enclosed with small seeds of the same species, and (b) The effect of seed size on female preference is influenced by female body size (and thus egg load).

To determine seed size and body size effects on oviposition preference for large vs. small seeds I used lines of beetles created by artificial selection to vary in body size. I used three selection lines (two replicates each) - UP beetles were selected to be large-bodied, CONTROL were the unselected ‘natural’ size beetles, and DOWN beetles were selected to be small. Details of this procedure will be presented elsewhere (J. Moya-Laraño and C. W. Fox, unpublished). In short, starting with the outbred ORACLE population artificial selection was imposed on female body size (two replicates each for the UP and DOWN lines). These selection lines were paired with unselected control lines propagated with randomly-chosen offspring (two replicate CONTROL lines). For the selected lines (UP and DOWN), 25 families of beetles were raised per generation, each with 10 offspring (250 total offspring), from which the 25 largest (UP lines) or smallest (DOWN lines) females were selected for the next generation. Emerging females were weighed within 12 h of adult emergence then paired with a randomly chosen male from the same line. Females were allowed to lay eggs until they laid one egg on >10 *A. greggii* seeds. 10 of these eggs were raised for the next generation. In the CONTROL lines two random eggs were selected from every female such that no selection was imposed on body size. Selection was imposed for nine generations after which beetles were raised for two generations of random mating (within lines, not between lines). At the end of selection, UP beetles were 30% larger than the CONTROL beetles and DOWN beetles were 40% smaller than CONTROL beetles.

Pairs of virgin beetles from each line (n = 100) were enclosed in 35 mm Petri dishes (one pair per dish) with two seeds of *Acacia greggii* of either one of the following three treatments: (a) two large seeds, (b) one large seed and one small seed, or (c) two small seeds. Seeds were sorted with a sieve. Large seeds averaged 2039 ± 10.3 mg and small seeds averaged 771.2 ± 5.6 mg. Dishes were inspected and eggs counted at 12 h intervals until at least 10 eggs were laid by each female.

To determine the effects of mixed treatments in fecundity and in the time to lay the first egg, I performed ANOVAs. Acceptability, measured as the proportion of females laying at least one egg on each seed size was evaluated with a logistic regression. All analyses were performed in SAS (SAS Institute 1985).

3.2.5.2 Results

Overall, when presented with one large and one small seed simultaneously, females from all lines laid more eggs on the large seed than on the small seed (Figure 3.3a; seed size effect $F_{1,349}$=413.1; P<0.0001). This pattern was very different when females were enclosed with two large or two small seeds - there were no significant differences in the number of eggs laid (Figure 3.3b; $F_{1,346}$=0.72; P=0.39); Larger females laid on average more eggs than medium and small females (Average number of eggs laid on large and small seeds: FUP = 11 and 3 respectively, CONTROL = 10 AND 3 respectively and FDOWN = 8 and 3 respectively; $F_{2,349}$ = 13.93; P<0.0001) and in the one seed size treatment (Average number of eggs laid on two large and two small seeds: FUP = 7 and 7 respectively, CONTROL = 7 and 6 respectively and FDOWN = 6 and 5 respectively; $F_{2,345}$=13.93; P<0.0001).

When presented with one large and one small seed simultaneously, a greater proportion of females from all lines laid at least one egg in larger than in smaller seeds. Thus, females from all lines show significantly lower acceptability to lay eggs on small seeds (Figure 3.4a; X^2_1=15.46, P=0.008); when enclosed with two small seeds acceptability is not significantly different from acceptability of two large seeds (Figure 3.4b; X^2_1=0.0010, P=0.97).

Overall, when presented with one large and one small seed simultaneously, females from all lines took a significantly different time to lay eggs on the small seed than in the large seed in the mixed treatment (Figure 3.5a; $F_{1,312}$=9.23; P=0.0024), but nor when they were enclosed only with two large or two small seeds (Figure 3.5b; $F_{1,345}$=1.45; P=0.23). I also detected a significant line effect when females were enclosed in the mixed treatment ($F_{2,313}$=5.45; P=0.0047), but the pattern is unclear.

These results indicate that females prefer to oviposit on large over small seeds when finding a mixed environment. Females laid more eggs on large seeds in the mixed-seed treatment. When comparing large vs. small seeds in no-choice treatments, females laid the same number of eggs on both sizes of seeds, the proportion of females accepting to lay at least one egg on each seed size and the time to lay eggs did not vary significantly. Regarding our second hypothesis, that the effect of seed size on female preference is influenced by female body size, I found that smaller females lay fewer eggs on small seeds, but I did not find a seed size-by-line interaction that would indicate a differential response of lines to seed size. Thus, our second hypothesis is rejected.

3.2.6 Experiment 3: Female preferences for seeds of varying species, size and quality

3.2.6.1 Methods

I tested the hypotheses that (a) females from different populations and adapted to different host species will prefer their native hosts over the alternative host, versus (b) females will prefer the larger-seeded host regardless of which host is their native host.

Pairs of virgin beetles from three populations (Anapoima, Del Rio and Oracle) were mated and enclosed in a 35 mm Petri dish (one pair per dish) with two seeds per dish in one of the following combinations: (a) one large and one small *A. greggii* seed, (b) one large *A. greggii* seed and one *Ps. guachapele* seed, or (c) one small *A. greggii* seed and one *Ps. guachapele* seed (N = 30 pairs per treatment per population). Each dish was inspected for eggs every 12 h until the female laid ≥ 10 eggs.

As in experiment two, to determine the effects of treatments in fecundity and in the time to lay the first egg, I performed ANOVAs. Acceptability, measured as the proportion of females that laid at least one egg on each seed size/type was evaluated with a logistic regression. All analyses were performed in SAS (SAS Institute 1985).

3.2.6.2 Results

Females from all three populations laid more eggs on seeds of *A. greggii* than on seeds of *Ps. guachapele* (Treatment large *A. greggii – Ps. guachapele,* figure 3.6a, $F_{1,167}$=683.9, P<0.0001; treatment Small *A. greggii – Ps. guachapele*, figure 3.6b, $F_{1,166}$=232.1, P<0.0001) and on large than in small *A. greggii* seeds (Figure 3.6c;$F_{1,171}$=195.35, P<0.0001). I also found a significant population effect when females were enclosed with a large *A. greggii* and a *Ps. guachapele* seed ($F_{2,167}$=4.14, P=0.017). Only one female out of 60 laid one egg on *P. guachapele* (Figure 3.6a).

Overall, a larger proportion of females from all populations accept to lay at least one egg on *A. greggii* than in *Ps. guachapele* (Seed type effect for the Large *A. greggii - Ps. guachapele* treatment, Figure 3.7a: X^2_1=38.32, P< 0.0001; small *A. greggii-Ps guachapele* treatment, Figure 3.7b*:* X^2_1=25.7, P<0.0001). Surprisingly, females from Oracle and Del Rio were less likely to reject *Ps. guachapele* seeds than were females from Anapoima and rejection of *Ps. guachapele* seeds were greatest when the alternate seed for oviposition was a large *A. greggii*. (Average proportion of females laying on *Ps guachapele*: Del Rio = 0,25, Oracle = 0.37, Anapoima = 0.034). In addition I found a significant interaction population by seed in the treatment small *A. greggii – Ps. guachapele*: X^2_2=12.17, P=0.0023). A similar proportion of females accept large and small seeds of *A. greggii* in this treatment (Figure 3.7c; X^2_1=2.47, P=0.11).

Females delayed oviposition on seeds of *Ps. guachapele* longer than they delayed oviposition on seeds of *A. greggii* (Treatment large *A. greggii – Ps. guachapele*, Figure 3.8a, $F_{1,168}$=70.34, P<0.0001; treatment Small *A. greggii – Ps. guachapele*, Figure 3.8b, $F_{1,165}$=15.55, P=0.0001). However, this delay in oviposition on *Ps. guachapele* was greater when the alternate host was a large *A. greggii* seed than when the alternate host was a small *A. greggii* seed (Figure 3.8). Anapoima beetles also took longer to start ovipositing irrespective of seed species or size. These results are consistent with the results of the rejection of seeds – females prefer to oviposit on large *A. greggii* seeds, followed by small *A. greggii* seeds and lastly *Ps. guachapele* seeds. Significant population by seed species interaction show that females responded differently to each seed type (Treatment large *A. greggii – Ps. guachapele* $F_{5,168}$=23.77, P<0.0001; treatment Small *A. greggii – Ps. guachapele* $F_{5,165}$=8.19, P<0.0001).

Thus, these results confirm the hypothesis that, regardless the native host, females prefer larger *A. greggii* seeds, followed by small *A. greggii* seeds and last *Ps. guachapele* seeds. They also show that a trade-off between local adaptation and use of alternative hosts is dependent on factors such as the quality and size of the alternative host compared with the quality and size of the native host.

3.2.7 Experiment 4: Egg size plasticity in response to host species, size and quality.

3.2.7.1 Methods

Previous studies have shown that Arizona populations of *S. limbatus* lay larger eggs on seeds of *Pa. florida* than on seeds of *A. greggii*, and that Colombian populations lay larger eggs on seeds of *Ps. guachapele* than on seeds of *A. greggii*. Here I simultaneously compare Colombian and Arizonan beetle responses to all three hosts and test the hypothesis that beetles from these two regions differ in their response (egg size plasticity) to these three host species. *A. greggii* and *Pa. florida* are hosts used by the USA populations and *Ps. guachapele* is a host used by the Colombia populations.

Emerging females were weighed within 24 of their emergence from their rearing seed. Prior to mating, to ensure that eggs are matured in contact with seeds of the test host, virgin females from the four populations, Anapoima, Melgar, Del Rio and Oracle, were individually enclosed for 48h in a 35 mm Petri dish (one female per dish) with eight seeds of their treatment host. Females were then mated to virgin males (N = 90 pairs per population) and transferred to 35 mm Petri dish (one pair per dish) containing three seeds of either *A. greggii*, *Ps. guachapele* or *Pa. florida* (N= 30 pairs per seed host per population). The former two of these hosts are natural hosts for beetle populations in Arizona whereas the third (*Ps. guachapele*) is a natural host for beetle populations in Colombia.

24h after mating seeds were inspected for eggs. If all three seeds had eggs, then the beetles were discarded. Dishes in which females had not laid eggs on all three seeds were inspected every 12h until eggs had been laid on all three seeds. Eggs were allowed to hatch (larvae burrow into the seed under the egg) then frozen at -20^{o}C until measured.

I measured the length of one randomly chosen egg (at 55x) from each of the three seeds for each female. The average length of these three eggs was treated as a single data point for each female. I used analysis of variance to test for the effects of population and treatment (oviposition host) on egg size. All analyses were conducted in SAS (SAS Institute, 1985).

3.2.7.2 Results

As has been found in other studies with *S. limbatus*, female body mass influenced egg size (analysis of covariance, $F_{1,212}$=12.36*;* P=0.0005). There was no significant difference between populations (within countries) in the size of eggs that they laid [population (nested within country) effect, $F_{3,212}$=1.15, P=0.32]. Females from all four populations laid larger eggs on seeds of both *Ps. guachapele* and *Pa. florida* than on seeds of *A. greggii* (Figure 3.9; host effect, $F_{2,212}$=43.1, P<0.0001). However, the magnitude of plasticity was different for Colombian and Arizona populations - Colombian females laid the smallest size eggs on *A. greggii* and larger eggs on both *Ps. guachapele* and *Pa. florida*, but the size of eggs that these females laid did not differ between *Ps. guachapele* and *Pa. florida* (Difference between means, tukey test = 0.004, no significant at 0.05 level). Arizona females also laid the smallest eggs on *A. greggii* and larger eggs on both *Ps. guachapele* and *Pa. florida*, but eggs laid on *Pa. florida* were significantly larger than eggs laid on *Ps. guachapele* (Figure 3.9; Difference between means, tukey test = 0.029, significant at 0.05 level); population nested within country-by-host effect, $F_{5,212}$=6.26, P<0.0001).

These results confirm our hypothesis that females from divergent clades of the geographic range of *S. limbatus* adjust the size of their eggs in response to the species of seed on which they lay those eggs but that the shape and magnitude of this response differs between Colombian and Arizona populations.

3.3 Discussion

Beetles from all populations show greater preference for large *A. greggii* seeds, followed by small *A. greggii* seeds and lastly seeds of *Ps. guachapele*. Females laid more eggs on larger seeds, and distributed their eggs more uniformly on seeds bearing fewer conspecific eggs. In chapter two I demonstrated that larval performance was greater for all populations tested here when reared on *A. greggii* seeds than when reared on *Ps. guachapele* seeds. Thus, females prefer to lay eggs on the species on which performance is greatest, consistent with the oviposition preference – offspring performance hypothesis of Jaenike (1978) and with the preference for larger and better quality seeds. Concordance between oviposition preference and offspring performance has been observed in some species of herbivorous, of predaceous and of parasitoid insects (Tauber and Tauber 1987, Poore and Steinberg 1999, Sadeghi and Gilbert 1999, Heisswolf et al. 2005). However there are cases in which preference for hosts is driven by other factors such as finding of enemy free space – even if the preferred host is not the best for offspring development, it enhances its survivorship by protecting them from natural enemies (Bernays and Graham 1988, Ballabeni et al. 2001, Lill et al. 2002). The influence of natural enemies on host use by the Colombia and Arizona populations of *S. limbatus* is currently under study.

Females from all populations tested here preferred to oviposit in *A. greggii,* a host available in nature only for the Arizona populations. According with the deme formation hypothesis (Edmunds and Alstad 1978) selection on the ability to distinguish hosts used in nature should reduce the ability to distinguish among alternative hosts not normally encountered in nature (Thompson 1996, Van Zandt and Mopper 1998, Agrawal 2000). Results from the third experiment do not support this hypothesis. Instead, I found that seed size and seed quality are the major determinants of variation in oviposition preference among these populations locally adapted to different hosts.

In addition to changes in female fecundity associated with changes in size and quality of the seeds to which they have access, I found that acceptability (the proportion of females that laid at least one egg on each seed size/type) of small and poor quality hosts is dependant of the availability of alternate hosts. Females that had a small *A. greggii* seed and a *Ps. guachapele* seed available for ovipisition laid more eggs on the small *Ps. guachapele* seed than did females that were enclosed with a large *A. greggii* seed and a *Ps guachapele*. This indicates that the acceptability of hosts is dependant of the availability and quality of alternate hosts. In another seed beetle for which host discrimination is well studied, *Callosobruchus maculatus*, female oviposition decisions follow a threshold model in which the acceptance threshold for a host is adjusted by experience gained during the egg laying process showing that as females become more host limited they reduce their aversion to ovipositing on the less preferred host (Horng et al. 1999). In my case, the quality of the alternate seed (large vs. small *A. greggii* seeds) was a function of the size of these seeds relative to the *Ps. guachapele* seed.

Females of *S. limbatus* assess the quality of the seed for oviposition based on seed size, nutritional content, toxicity and number of eggs previously laid upon it. Females also asses the number of conspecific eggs already present on the seeds and distribute their own eggs in a way that minimizes the effect of larval competition on survivorship. As the number of conspecific eggs on seeds increases females are less accepting of those seeds and distribute their eggs less evenly among the available seeds. Experiments with *C. maculatus* show a similar pattern. Females of this species distribute their eggs uniformly among seeds (Mitchell 1975) and are able to discriminate among seeds with small differences in egg number, laying preferentially on seeds with lower egg densities (Messina and Renwick 1985a).

All populations studied here showed similar responses to small seeds and to *Ps. guachapele* seeds – acceptability of *Ps. guachapele* seeds was higher when the alternate host was a small rather than a large *A. greggii* seed, and all populations exhibited egg size plasticity in response to low quality and/or toxic hosts. This suggests that female preference is relatively conserved across the broad distribution of the species. Populations from Colombia and Arizona represent very divergent clades on the *S. limbatus* phylogeny (Morse and Farrell 2005a), and females adjust egg size in response to seed quality. These results are consistent with the hypothesis I proposed in chapter two, that egg size plasticity is an ancestral trait in *S. limbatus* that facilitates colonization of new hosts, allowing diversification of diet and range expansion. The importance of egg size plasticity in colonization of new hosts in *S. limbatus* has been demonstrated by Fox and Savalli (2000) in reference to colonization of Texas ebony, a widespread ornamental, by an *S. limbatus* population in Arizona. They showed that females exposed to *Pa. florida*, which produces seed coats resistant to larval penetration, lay larger eggs and change egg composition and thus increase larval survivorship on this host by 10-fold, relative to the survivorship of larvae hatching from eggs of females that never encounter seeds of *Pa. florida*.

In addition, the small body size of beetles in the Colombian populations may be a constraint on how large eggs can be in response to host quality – the size and the shape of the oviducts may limit the absolute size of eggs laid, regardless of the size favored by selection, in some insects. This may explain why, even though all four populations show egg size plasticity in response to their host species, Colombian populations are not as plastic as Arizona populations. Thus, although small body size has evolved in the Colombian populations in response to the small size of their host seeds, this small body size may constrain egg size plasticity and thus the ability of beetles to expand onto new hosts.

In conclusion, I demonstrated that irrespective of population origin, females of *S. limbatus* prefer to oviposit on large over small *A. greggii* seeds (Experiment two) and on the best quality seeds of *A. greggii* than on the smaller and low quality seeds of *Ps. guachapele.* Preference for the least preferred *Ps. guachapele* is dependant of the size of *A. greggii* and females from Anapoima laid the lower number of eggs on *Ps. guachapele* and had the lowest acceptability for this host (Experiment three). In addition to show higher preference for the larger host, results from these experiments agree with the hypothesis of a correlation preference performance (Jaenike 1978). Females preferred to oviposit in the host in which offspring performs better (Chapter two). Once females lay eggs on a selected seed, they lay more eggs on seeds with no eggs than on seeds bearing already eggs (Experiment one) and distribute eggs evenly among seeds available, though this evenly distribution of eggs decrease as eggs already on the seed increase (Experiment one). Females also exhibit egg size plasticity, laying larger eggs on the toxic *Pa. florida* and in the low quality *Ps. guachapele* seeds (Experiment four). However, body size may constrain how large eggs laid by Colombia females could be by an unknown mechanism. The size of oviducts may limit the size favored by selection. Thus, egg size plasticity, considered as an ancestral trait, may be a very important mechanism that favors host colonization and host expansion in *S. limbatus,* but body size may impose a constrain on how plastic organisms can be.

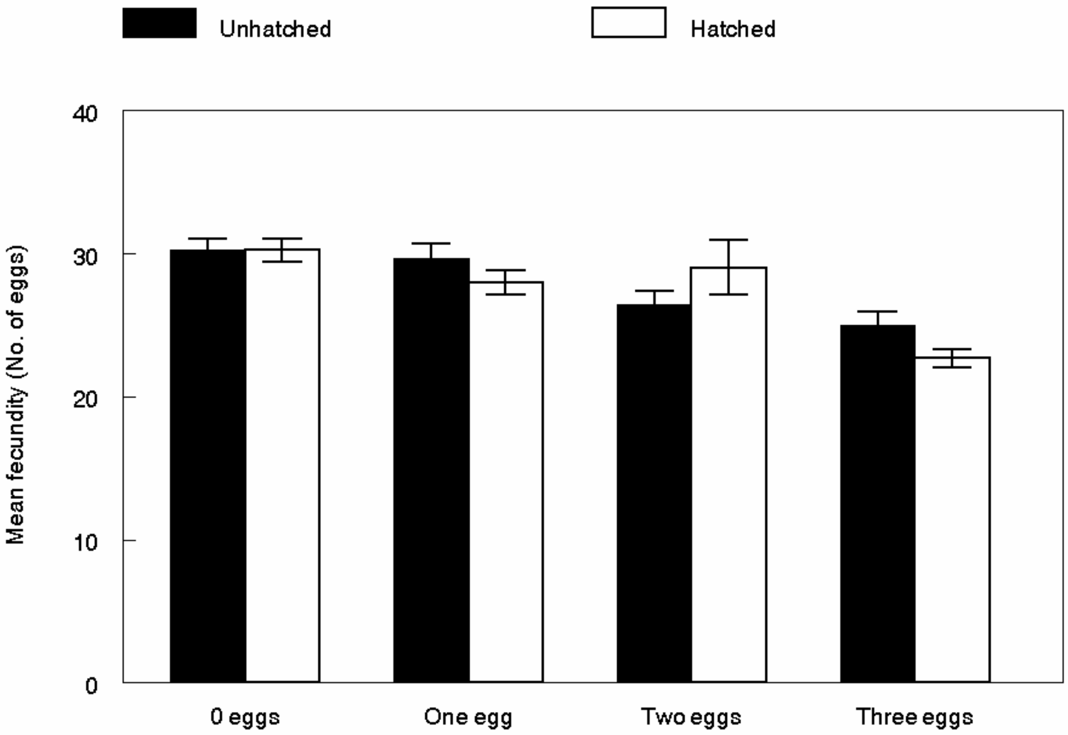

Figure 3.1: Mean fecundity of females on seeds with differential number of eggs already laid and of different developmental stages. Black bars: Eggs not hatched. White bars: Hatched eggs.

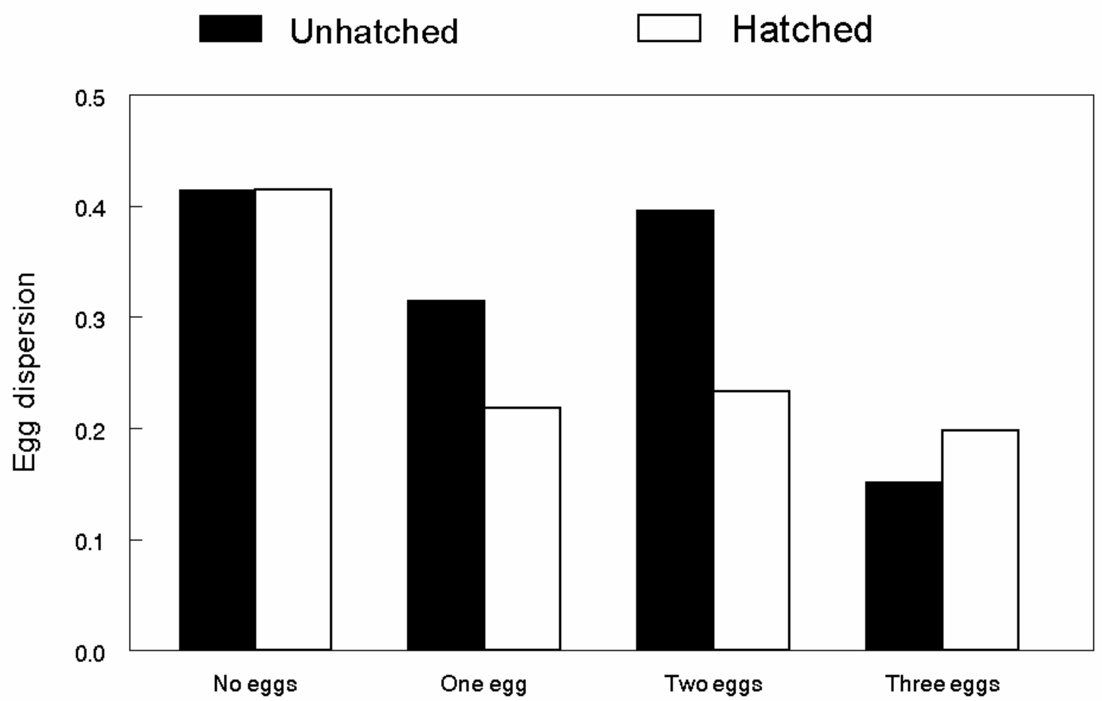

Figure 3.2: Distribution of eggs laid by females on seeds with differential number of eggs already laid and of different developmental stages. Black bars: Eggs not hatched. White bars: Hatched eggs. A more even distribution of eggs is represented by a higher egg dispersion value.

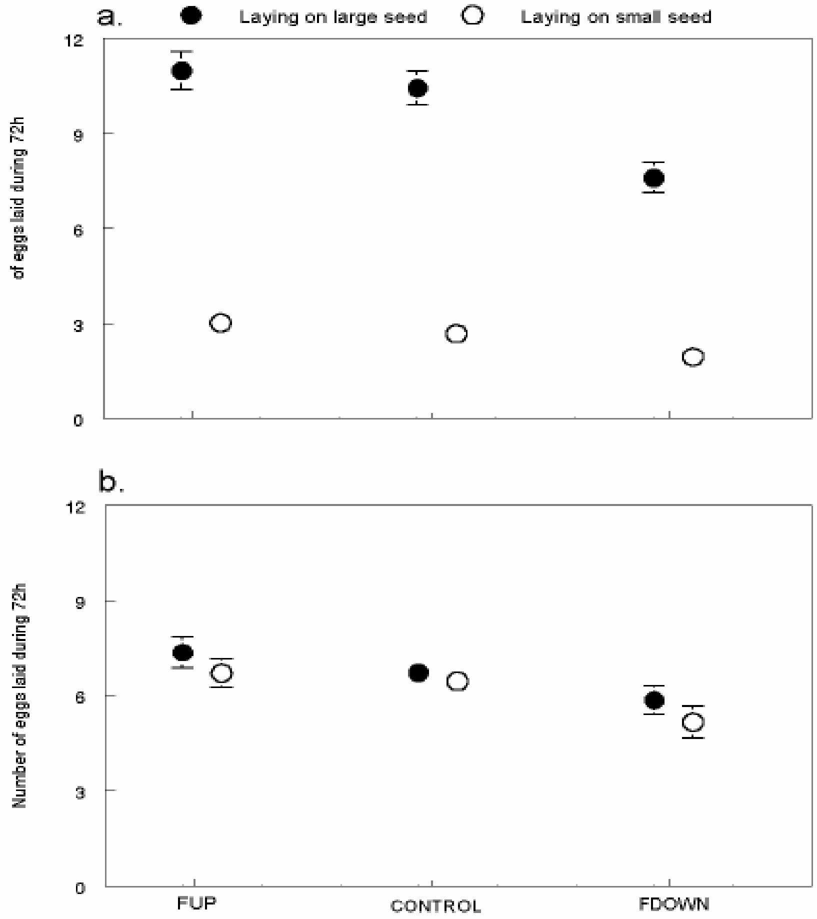

Figure 3.3: The number of eggs laid by female *S. limbatus* on large versus small seeds when presented (a) simultaneously with one large and one small *Acacia greggii*. (b) with two large or two small *Acacia greggii* seeds. FUP: Larger females, CONTROL: Medium size females, and FDOWN: Smaller females. Standard errors are smaller than the symbols in some cases.

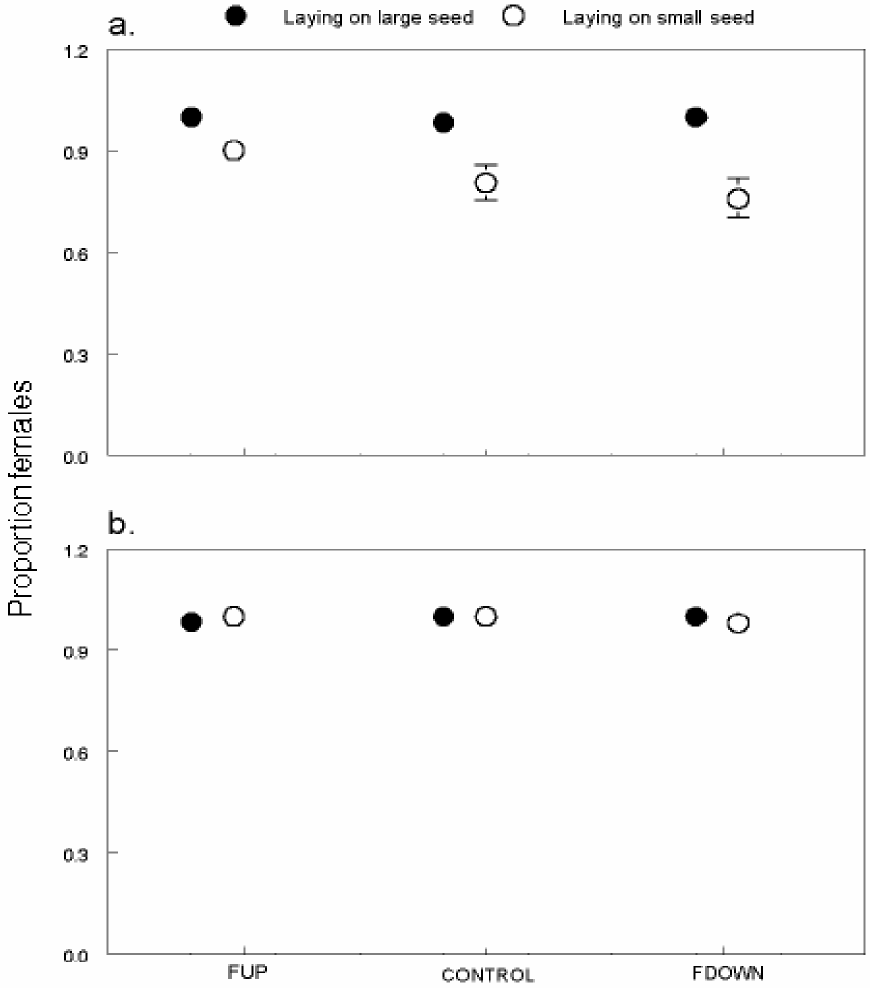

Figure 3.4: The proportion of female *S. limbatus* laying at least one eggs on large versus small seeds when presented (a) simultaneously with one large and one small *Acacia greggii*. (b) with two large or two small *Acacia greggii* seeds. FUP: Larger females, CONTROL: Medium size females, and FDOWN: Smaller females. Standard errors are smaller than the symbols in some cases.

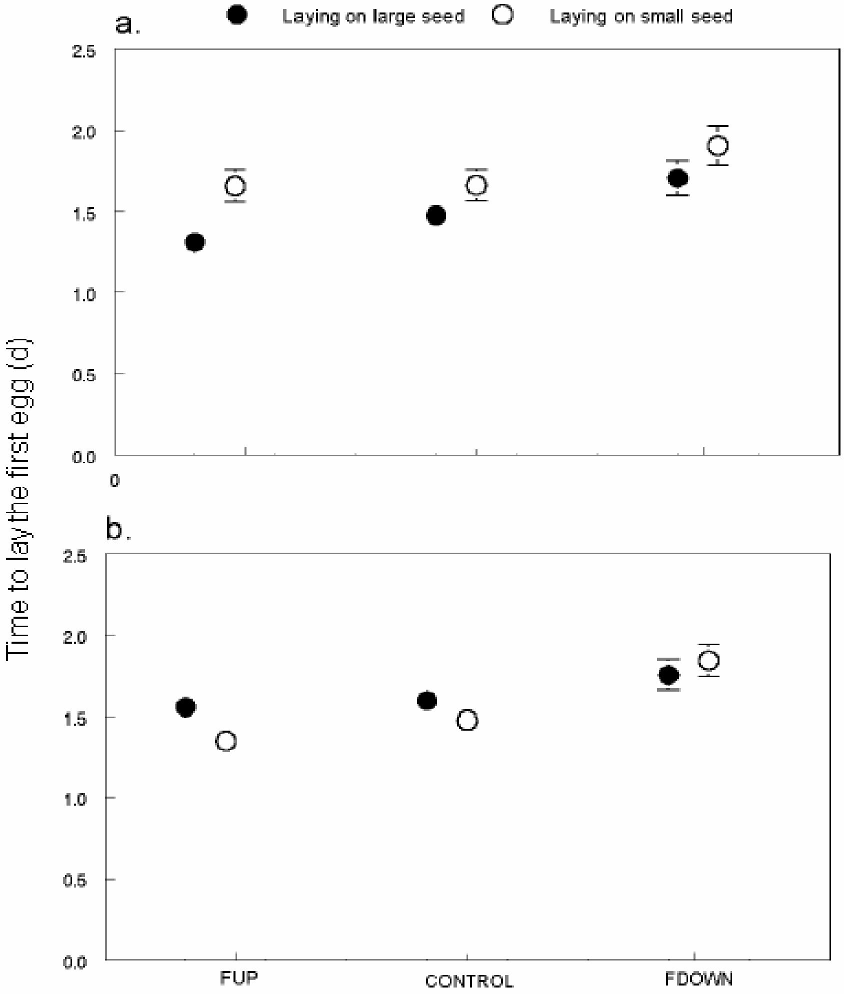

Figure 3.5: The time females *S. limbatus* last to lay the first egg on large versus small seeds when presented (a) simultaneously with one large and one small *Acacia greggii*. (b) with two large or two small *Acacia greggii* seeds. FUP: Larger females, CONTROL: Medium size females, and FDOWN: Smaller females. Standard errors are smaller than the symbols in some cases.

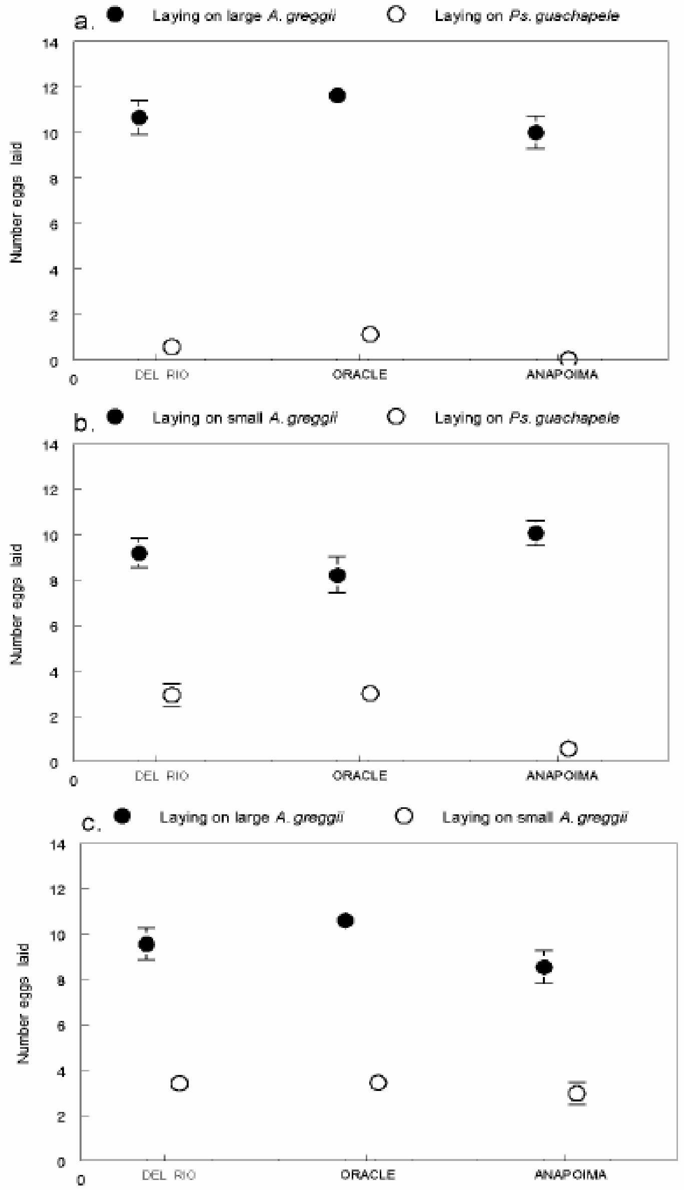

Figure 3.6: The number of eggs laid by female *S. limbatus* on seeds when presented simultaneously with (a) one large *Acacia greggii* and one *Ps. guachapele* (b) one small *Acacia greggii* and one *Ps. guachapele* and (c) one large and one small *Acacia greggii*; DEL RIO: Larger beetle size population, ORACLE: Medium size beetle population, ANAPOIMA: Smaller beetle size population. Standard errors are smaller than the symbols in some cases.

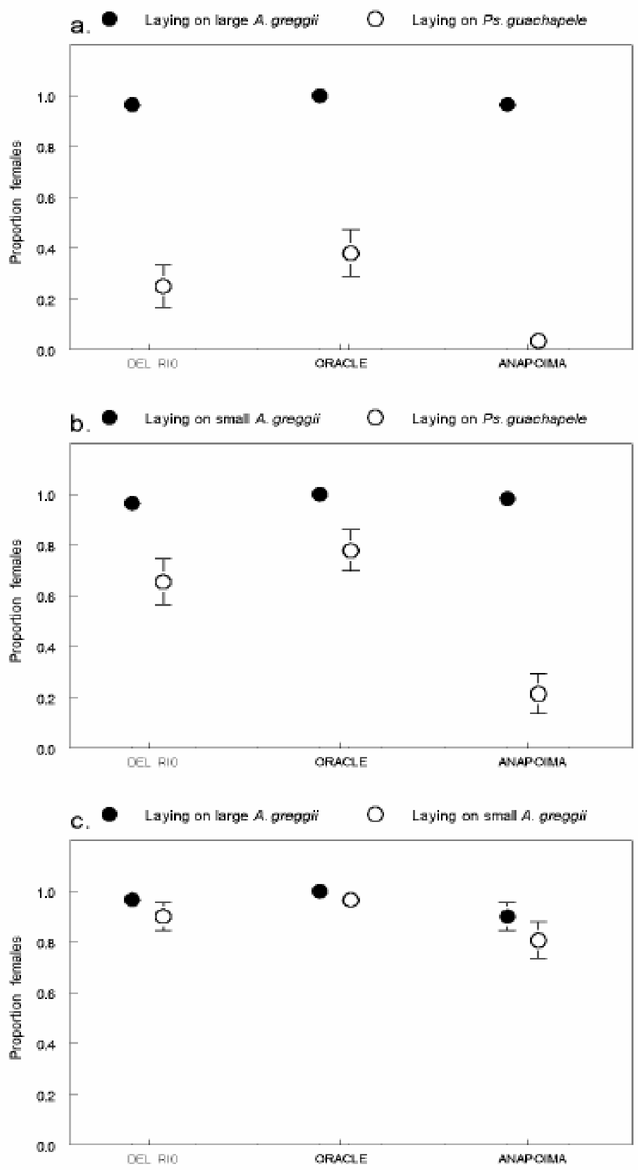

Figure 3.7: The proportion of females of *S. limbatus* that laid at least one egg on seeds when presented simultaneously with (a) one large *Acacia greggii* and one *Ps. guachapele* (b) one small *Acacia greggii* and one *Ps. guachapele* and (c) one large and one small *Acacia greggii*; DEL RIO: Larger beetle size population, ORACLE: Medium size beetle population, ANAPOIMA: Smaller beetle size population. Standard errors are smaller than the symbols in some cases.

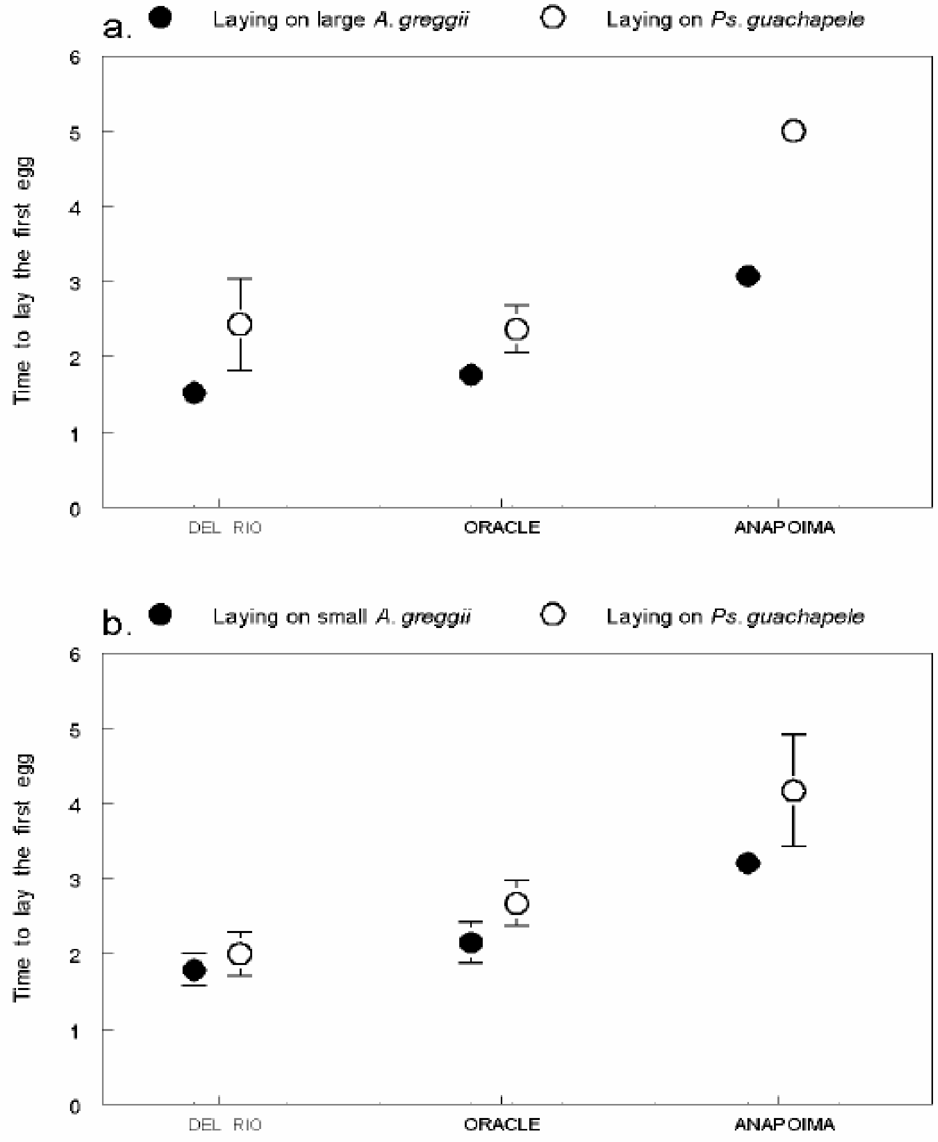

Figure 3.8: The time females of *S. limbatus* last to lay at least one egg on seeds when presented simultaneously with (a) one large *Acacia greggii* and one *Ps. guachapele* (b) one small *Acacia greggii* and one *Ps. guachapele.* DEL RIO: Larger beetle size population, ORACLE: Medium size beetle population, ANAPOIMA: Smaller beetle size population. Standard errors are smaller than the symbols in some cases.

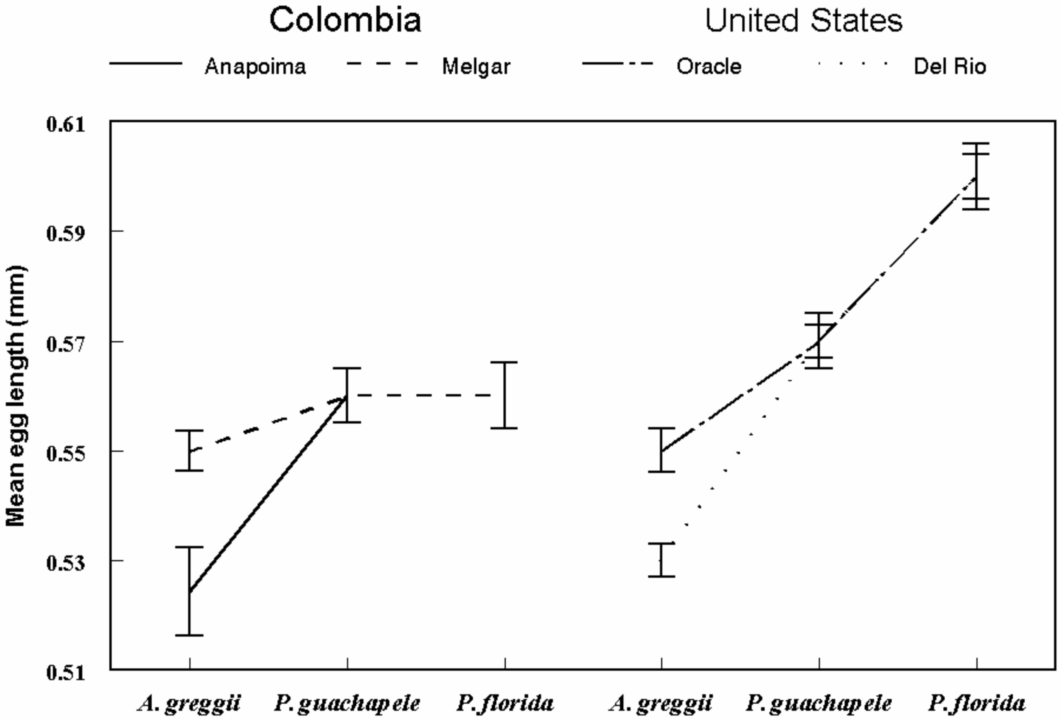

Figure 3.9: Changes in egg length in populations of *S. limbatus* of Colombia and the United States in response to three host seeds.

Chapter 4: Effects of seed size and insect size on the consequences of larval competition

4.1 Introduction

Competition for resources, such as space, mates, territory and food, is one of the major factors determining animal morphology and life histories. Many traits have evolved that confer increases in competitive ability, or that reduce the negative effects of competition on animal fitness (Pexton and Mayhew 2004). For insects developing on discrete resources, in which host size and hence larval resources are fixed at oviposition, intraspecific larval competition has particularly important consequences for individual fitness when organisms cannot move to new resource patches, and when larval competition is high (Hess et al. 1996). In scramble competing species, larvae reared at high density usually mature smaller (Bai and Mackauer 1992, Hardy et al. 1992, Mackauer and Chau 2001) and suffer higher larval mortality (Hess et al. 1996, Fox and Savalli 1998) but mature sooner (Ode et al. 1996, review in Roff 1992). For example, larvae of the seed feeding beetle, *Stator limbatus*, that develop at higher density are smaller and have lower survivorship than larvae that develop at low density (Fox et al. 1996).

Studies with some gregarious parasitoids, for which development is analogous to seed feeders that undergo scramble competition (Messina 2004), show that host size and quality are important determinants of life histories, to the extent, that host size has been proposed to constrain the evolution of clutch size (Hardy et al. 1992, Tsai et al. 2001). In both gregarious parasitoids and seed feeding insects, adult body size and development time increase with host size (Boivin and Lagace 1999, Mackauer and Chau 2001). For example, populations of the seed feeding beetle *S. limbatus* vary in adult body mass concordant with variation in host size (see chapter two); beetles from populations that use *Acacia greggii* are on average 49% larger (body mass) than beetles from populations that use the small seeds of *Pseudosamanea guachapele* (Chapter two). Moreover, components of host plant quality such as size, including nitrogen, carbon and secondary compounds, also affect oviposition behavior, fecundity, development time, offspring size, and a variety of offspring traits (Chapter three in this dissertation, Awmack and Leather 2002).

Adult body size is perhaps one of the major traits affecting the performance of organisms with important consequences for fitness (Price and Schluter 1991, Roff 1992), metabolic rates and even population growth (Savage et al 2003, Charnov and Gillooly 2004). It affects almost all aspects of the life cycle, and often has large effects on adult traits such as mate selection, fecundity and offspring size (e.g., egg size, and survivorship on Visser 1994, Savalli and Fox 1998). Given that large adults have larger progeny than their smaller conspecifics, their offspring requirements for development and survivorship will be higher, and thus, is expected that these requirements will be scarcer as competition increase, suggesting that density dependent effects on body size would be more deleterious to large organisms than to small ones.

Thus, it is expected that, in scramble competition systems with a fixed amount of resources for development, larger animals will be more susceptible to host size and to increasing competition than smaller animals. However, no previous studies have examined simultaneously the effects of host size, competition and body size on growth and life history traits in insects with resource limitation during development. A factorial experiment, varying simultaneously these factors, will allow to examine potential causal relationships and interactions among these components giving a better understanding of how clutch size, body size and host size may influence the evolution of life histories in organisms that use discrete hosts (Messina 2004).

Thus, the aim of this study was to determine the fitness consequences of body size of a seed-feeding beetle in response to simultaneous variation in larval competition and seed size. Specifically we asked: (1) Do the effects of larval competition vary with host size? and (2) Do larger beetles suffer greater fitness costs to being reared at high density or on small seeds relative to smaller-bodied beetles?

To address these questions we performed two sequential experiments. First, we simultaneously manipulated larval density (1-16 larvae per seed) and seed size (large, medium, and small seeds) and quantified the consequences of these manipulations for growth and survival of beetles from three natural populations that are adapted to different size hosts (seeds), and differ in adult body size. Second, we tested whether the observed variation among populations in their response to seed size and larval density is likely a consequence of variation in body size by repeating our experiment using lines of three different sized beetles obtained by artificial selection from a single population.

4.2 Materials and Methods

4.2.1 The Model Organism

S. limbatus (Horn) (Coleoptera: Chrysomelidae: Bruchinae) is a seed-feeding beetle with a broad distribution ranging from the northwest of Argentina in South America to the southwest of the United States in North America (Johnson and Kingsolver 1976, Johnson et al. 1989). Throughout its distribution *S. limbatus* feeds on seeds of about 80 species of legume trees, but populations at each locality usually have access to just a few species.

Females oviposit directly onto mature seeds. First instar larvae hatch from eggs, burrow into, and develop entirely inside seeds. Adults emerge around 28-30 days later at 28^{o}C. In the laboratory, oviposition in the southwestern United Sates desert populations starts 12-48 hours after emergence if mates and seeds are available. For the Colombian populations, oviposition starts about 48 hours after emergence if multiple mates, seeds and food are provided.

4.2.2 Source Populations and Colony Establishment

The three populations used for this study were collected at three localities from three different host species that produce different sized seeds. Beetles were collected from the small-seeded *Pseudosamanea guachapele* (Anapoima, Cundinamarca, Colombia, South America, 4°31'13''N; 74°32'22''W) in December 2002, from the medium size seeds of *Acacia greggii* (Oracle, Pinal Co., Arizona, United States, 32°36'39''N; 110°46'13''W) in August 2002, and from the large-seeded *Acacia berlandieri* (Del Rio, Texas, United States, 29°28'31''N; 100°59'21''W) in August 2003. *ORACLE* beetles are on average 8% smaller (body mass) than *DEL RIO* beetles, and *ANAPOIMA* beetles are 52% smaller than *DEL RIO* beetles. *A. greggii* seeds are 15-20% smaller than *A. berlandieri* seeds, and *P. guachapele* seeds are 60% smaller than *A. berlandieri* seeds. These field collected populations (*DEL RIO*, *ORACLE* and *ANAPOIMA*) differ in a variety of growth and life history traits other than mean body size. Many of these differences are likely a consequence of adaptation to different host species and to seeds of different size (Chapter two).

Mature seed pods were collected from at least 20 trees at each locality. These pods were opened in the laboratory and seeds bearing eggs were placed in individual Petri dishes inside a growth chamber at 28^{o}C, 15:9 light: dark. Emerging adults (>200 from each population) were used to establish laboratory colonies. Each colony was maintained in the lab at >100 families per generation at 28^{o}C, 15:9 light: dark. Because survivorship of all populations is very high on *A. greggii* seeds (Chapter two) all colonies were maintained on this host prior to beginning the experiment at least for nine generations. The use of a common host was necessary to eliminate host-associated maternal effects that could confound population differences in growth and body size (Fox et al 1996). All beetles in the colonies were raised to adult at one larva per seed.

4.2.3 Body Size Selected Lines

To confirm whether the variation in response to seed size and larval density observed among natural populations in our first experiment was potentially a consequence of variation among populations in their body size, we repeated our experiment using lines of beetles created by artificial selection to differ in mean body size. Lines were selected to be composed by large (*UP* line), small (*DOWN* line), or medium size (*CONTROL* line) beetles. They were all created from the *ORACLE* population and thus differences between the lines can only be a consequence of selection for differences in body size and not a consequence of different evolutionary histories with respect to seed size or larval density.

Details of creation of the selected lines will be presented elsewhere (J. Moya-Laraño and C. W. Fox, unpublished). In short, starting with the outbreed *ORACLE* population large and small beetles were created by imposing artificial selection on female body size (two replicates each of an *UP* and a *DOWN* line). These selection lines were paired with unselected control lines propagated with randomly-chosen offspring (two replicate *CONTROL* lines). For the selected lines (*UP* and *DOWN*) 25 families of beetles were raised per generation, each with 10 offspring (250 total offspring), from which the 25 largest (*UP* lines) or smallest (*DOWN* lines) females were selected for the next generation. Emerging females were weighed within 12 h of adult emergence, and then paired with a randomly chosen male from the same line. Females were allowed to lay eggs until they laid one egg on >10 *A. greggii* seeds. 10 of these eggs from each female were raised for the next generation. In the *CONTROL* lines two random eggs were selected from every female such that no selection was imposed on body size.

Selection was imposed for nine generations, after which beetles were raised for two generations without selection. At the end of selection, *UP* beetles were 30% larger than *CONTROL* beetles, and *DOWN* beetles were 40% smaller than *CONTROL* beetles.

4.2.4 Experimental Design

We set up two independent factorial experiments. Both experiments were identical except in the study populations we used. Experiment 1 compared the three natural populations of *S. limbatus* that differ in body size and in their natural seed size/host species (*DEL RIO, ORACLE, ANAPOIMA*). Experiment 2 compared the three artificially selected lines of beetles that differed in body mass but were created from the single *ORACLE* population (*UP, CONTROL, DOWN* lines).

Pairs of beetles from each population/line were allowed to oviposit on clean seeds of three different sizes (large, medium and small) and were reared to adult at six different densities (1, 2, 4, 8, 12 and 16 eggs per seed). Large and medium-size seeds were *A. greggii* seeds sorted by diameter using a sieve. Average mass for large and medium seeds were 2039 ± 10.3 mg and 771.2 ± 5.6 mg, respectively. Smaller *A. greggii* seeds were typically aborted/abnormal and thus were not used. For the smallest seed class seeds of *P. guachapele* were used. Thus, small seeds differed from large and medium seeds in both size and species. Average mass of these seeds was 345.8 ± 2.94 mg.

12 h after emergence, virgin females were each mated to a virgin male (from the same population/line) and randomly assigned to a seed size treatment. Each pair of beetles was confined with 1, 2, 4 or 8 clean seeds to obtain 1, 2, 4, 8, 12 or 16 eggs per seed (see details below). Seeds were inspected every 24h until the pre-defined number of eggs per seed were laid. Excess eggs laid on the seeds were scraped off with a pair of forceps. Seeds bearing eggs were placed in a growth chamber at 28 ^{o}C, L: D 15:9, at one seed per dish.

To manipulate egg density, the number of seeds provided to mated pairs was manipulated. Pairs of beetles were provided either with (a) 8 seeds (20 pairs per seed size) to obtain a density of one egg per seed, (b) 4 seeds (10 pairs per seed size) to obtain two eggs per seed, (c) 2 seeds (10 pairs per seed size) to obtain four eggs per seed, (d) 2 seeds (10 seeds per seed size) to obtain six eggs per seed, (e) 1 seed (10 pairs) to obtain eight eggs per seed, (f) 1 seed (10 pairs) to obtain 12 eggs per seed, and (g) 1 seed (10 pairs) to obtain 16 eggs per seed. The last three larval densities were created only on large seeds because inspection of clutch sizes on seeds collected in the field indicated that females in nature rarely lay these densities of eggs on small and medium-sized seeds. Only eggs that hatched were counted as part of the larval density treatments.

In summary, a total of 4 density treatments (1, 2, 4 and 6) were established per population/line on the small and medium size seeds, whereas 7 density treatments (1, 2, 4, 6, 8, 12 and 16) per population/line were set up on the large seeds. I raised larvae from a total of 5,040 eggs for Experiment 1 (comparing the natural populations) and approximately 10,080 eggs, evenly divided amongst the two replicate sets of lines, for Experiment 2 (comparing the selected lines).

I recorded larval survivorship (from egg hatch to adult emergence) and development time of all surviving beetles. All emerging beetles were weighed on electronic balances (Mettler Toledo AT261 Delta range) to 0.01 mg within 12h of emergence.

4.2.5 Analyses

Logistic regression was used to test for population, seed size, and larval density effects on larval survivorship. ANOVA (Type III sums of squares) was used to examine the effect of population or line (Experiments 1 and 2, respectively), seed size, and larval density on body mass and development time. Because I had two replicates from each selected line (Experiment 2), I included a replicate effect in the ANOVAS. Least Squares Means (LS Means) were used to estimate effect sizes of individual model parameters; these are presented in the text to show the size of individual effects. However, all figures present actual means and not LS Means. All analyses were performed in SAS 8.2 (SAS Institute,
Cary, North Carolina, USA).

Because the highest larval density treatments (8, 12 and 16 eggs per seed) were created only for large seeds, I performed two sets of analyses. In the first set I included the densities created for all seed sizes (1 to 6 eggs per seed) across all populations/lines. For the second set of analyses I included only large seeds but consider all larval densities. In all cases the results from both set of analyses are consistent with each other; I thus present only the results of the first set of analyses.

4.3 Results

4.3.1 Experiment 1: Seed Size and Larval Density Effects on Growth and Survival in Natural Populations Differing in Body Mass

In this first experiment I compared the three natural populations of *S. limbatus* that differ in body mass, *DEL RIO* (largest beetles), *ORACLE* (intermediate-sized beetles) and *ANAPOIMA* (smallest beetles).

4.3.1.1 Hatch-to-Adult Survivorship

Hatch-to-adult survivorship varied with seed size (X^2_2= 259.4, P<0.0001), with larval density (X^2_5 = 58.4, P<0.0001) and among populations (X^2_2= 9.47, P=0.0087). Overall, survivorship decreased as seed size decreased, though the difference in survivorship was greater between large/medium and small seeds than between large and medium size seeds (Figure 4.1;average survivorship, 0.94on large, 0.94 on medium and 0.68 on small seeds). Survivorship also decreased with increasing larval density for all populations, but the magnitude of the larval density effect depended on seed size (seed size-by-larval density interaction, X^2_{10}=24.1, P=0.007) – the effect of increasing larval density was greatest for larvae in the smallest seed (average survivorship at 5-6 larval density on large seeds =0.92, on medium seeds =0.79 and on small seeds =0.35) and it also depended of population (population-by-larval density interaction, X^2_{10}=23.35, P=0.0095).

4.3.1.2 Hatch to Adult Development Time

Development time varied with seed size ($F_{2,810}$=2.05, P<0.0001) and with larval density ($F_{5,810}$=9.65, P<0.0001), but not among populations ($F_{2,810}$=2.05, P=0.0804). However, when including in the analysis development time at densities higher than 6 larvae per seed or all densities, there were significant differences among populations ($F_{2,79}$=3.99, P=0.022 for densities higher than 6 and $F_{2,957}$=3.24, P=0.04 including all densities). Overall, development time was longer on small seeds than on either large or medium seeds, but there was no difference in larval development time between large and medium size seeds (Figure 4.2; LS means: large seeds 26.4 ± 0.1d; medium seeds 27.2 ±0.1d; small seeds 32.0 ± 0.2d). Larval development time was also shortest at high density and longest at low density (Figure 4.2).

Neither the effect of seed size nor the effect of larval density varied among populations (i.e., no significant population-by-density or population-by-seed size interactions; P>0.05 for each). However, there was a significant three-way population-by-seed size-by-larval density interaction (Figure 4.2; $F_{19,810}$=1.61, P=0.047) – development time of the *DEL RIO* and *ANAPOIMA* beetles decreased as density increased from 1 to 4 larvae per seed in the small seeds, but it did not changed or increased between 4 and 6 larvae per seed in DEL RIO and ANAPOIMA respectively. Meanwhile, development time for the medium sized ORACLE beetles decreased with increasing larval density from 1 to 6.

With regard to the differential response to larval competition on different seed sizes, we did no found a significant seed size x larval density interaction ($F_{10,810}$=1.19, P=0.29).

4.3.1.3 Emergence Body Mass

Body mass varied with seed size ($F_{2,810}$= 321.5, P<0.0001), larval density ($F_{5,810}$=30.3, P<0.0001), and among populations ($F_{2,810}$=374.4, P<0.0001). Overall, beetles emerged largest when developing in the largest seeds and at the lowest density, and emerged smallest when developing in the smallest seeds and at highest densities (Figure 4.3). The effect of seed size on body mass varied among populations (population-by-seed size interaction $F_{4,810}$=42.2, P<0.0001) – *DEL RIO* beetles (the largest-bodied population) were most affected by seed size and the *ANAPOIMA* beetles were the least affected by seed size (Proportional reduction in body mass between large and small seeds, *DEL RIO*: 41.1%, *ORACLE*: 40.9%, *ANAPOIMA*: 21.2%). Likewise, the effect of increasing larval density varied among populations (population-by-larval density interaction, $F_{10,810}$=6.0, p<0.0001) – *DEL RIO* beetles were the most affected by larval density (Proportional reduction in body mass between 1-2 and 5-6 larval densities *DEL RIO*: 13.9%) and *ANAPOIMA* beetles were the least affected by larval density (Proportional reduction in body mass between 1-2 and 5-6 larval densities: 3.3%).

Males were larger than females ($F_{1,810}$=13.0, P=0.0100), though the degree of sexual dimorphism varied among populations (population-by-sex interaction; $F_{1,810}$=3.52, P=0.03010; Sexual size dimorphism (male size-female size)/male size: *DEL RIO* =0.044, *ORACLE* =0.021, *ANAPOIMA* =0.14). The degree of sexual dimorphism did not vary with either seed size or larval density (non-significant sex-by-density and sex-by-seed size interactions, P>0.05).

Regarding the differential response to larval competition on different seed sizes, we found that the response was not independent of population (population by larval density effect $F_{19,810}$=1.82, P=0.017) and that the effect of larval density was not independent of larval density (seed size by larval density effect $F_{10,810}$=5.5, P<0.0001). For example, *ANAPOIMA* beetles did experience a significant reduction in body mass when raised at higher densities, even though the differences in body mass were small compared with the reduction experienced by the largest *DEL RIO* and *ORACLE* beetles.

4.3.2 Experiment 2: Seed Size and Larval Density Effects on Growth and Survival in Selection Lines

4.3.2.1 Hatch-to-Adult Survivorship

As with the natural populations, hatch-to-adult survivorship varied with larval density (X^2_{18} = 13.02, $P<0.0001$), and among the body size lines (X^2_2= 13.02, $P=0.001$), (Figure 4.4). Larger beetles experienced significant decreased survivorship as seed size decreased and as larval density at each seed size increased, while medium and smaller beetles presented overall significant reduction in body mass only from large or medium seeds to small seeds (line-by-larval density effect X^2_{29}= 47.61, $P=0.001$). In all cases, seed size effects on larval survivorship were highly significant when doing pair wise comparisons (large vs. medium *A. greggii* seeds $X^2_{1=}$ 99.53, $P=0.0002$; large *A. greggii* vs. *P. guachapele* $X^2_{1=}$13.4, p=0.0003; medium *A. greggii* vs. *P. guachapele* $X^2_{1=}$ 86.32, p<0.0001), (Figure 4.4).

4.3.2.2 Hatch to Adult Development Time

As with the natural populations, development time varied with seed size ($F_{2,1648}$= 379.4, $P<0.0001$) and larval density ($F_{5,1648}$=4.45, $P=0.0005$); in contrast with the natural populations there was also variation among the selected lines when considering densities between 1 and 6 (Figure 4.5; $F_{5,1648}$= 5.50, $P<0.0001$). As in Experiment 1, development time was longer in small seeds and shortest in large seeds with a small difference in development time between large and medium size seeds (Figure 4.5; LS means, large seeds, 28.3 ± 0.097; medium seeds, 28.8 ± 0.1; small seeds, 34.4 ± 0.12). Also, as observed in Experiment 1, development time was longest at low density and shortest at high density. In contrast to our results for the natural populations, the beetles from the three body size lines responded differently to larval competition ($F_{25,1648}$=2.19, $P=0.0006$) and the effect of larval density varied with seed size ($F_{10,1648}$=2.01, $P=0.03$). We also detected a significant 3-way interaction (line-by-seed size-by-larval density interaction; $F_{249,1648}$=2.10, $P<0.0001$).

Overall, the effect of larval density on development time was generally smaller than observed in Experiment 1, the patterns were similar for single effects but in this case the effect of larval density varied among lines and with seed size.

4.3.2.3 Emergence Body Mass

As in Experiment 1, body mass varied with seed size ($F_{2,1647}$=1052.8, P<0.0001), with larval density ($F_{5,1647}$=50.75, P<0.0001) and among the selected lines ($F_{5,1647}$=72.14, P<0.0001), (Figure 4.6). As observed in Experiment 1, beetles were larger when developing in the smallest seeds and at higher densities. In contrast with Experiment 1, the medium size beetles (the *CONTROL* line) were most affected and the smallest beetles (*DOWN* lines) were least affected by a decrease in seed size (proportional reduction in body mass between large and small seeds, *UP*: 45.7%, *CONTROL*: 49.5%, *DOWN*: 42.2%; line-by-seed size interaction, $F_{10,1647}$=7.8, P<0.0001). The *CONTROL* beetles were also the most affected and the *DOWN* beetles were least affected by larval density (proportional reduction in body mass between 1-2 and 5-6 larvae per seed, *UP*: 18.1%, *CONTROL*: 18.8%, *DOWN*: 7%; line-by-larval density interaction, $F_{25,1647}$=3.96, P<0.0001) thought differences between the *CONTROL* and *UP* lines were small. I also detected in this experiment a significant line-by-seed size-by-larval density interaction ($F_{49,1647}$=1.8, P=0.0007) – larval density effects were generally greatest on medium size seeds for the *UP* lines, and on small seeds for the *CONTROL* and *DOWN* lines (significant interaction seed size-by-larval density: $F_{10,1647}$= 6.23, P<0.0001), (Figure 4.6).

Males were larger than females ($F_{1,1647}$= 19.45, P<0.0001) but, in contrast with the natural populations, all lines responded in a similar way ($F_{5,1647}$=0.73, P=0.60).

4.4 Discussion

With this study I tested (1) whether body size affects the consequences of variation in larval competition on different sized hosts, and (2) whether the effects of larval competition vary with host size. Regarding the first question I expected that larger beetles would suffer greater fitness costs than smaller beetles when developing at higher densities and in smaller seeds (significant population x larval density and population x seed size effects). Regarding the second question, I expected that the effects of larval competition on fitness would be greater on smaller than on larger seeds (significant larval density x seed size effect).

The first prediction was partially confirmed by the experiments. I observed a reduction in body mass and survivorship in response to increased larval density and seed size in all three *S. limbatus* populations. However the larger *DEL RIO* beetles were more affected than the smaller *ANAPOIMA* beetles indicating that the amount of plasticity varied significantly among different sized beetles. This highly significant interaction population-by-larval density for body mass and survivorship was evident also for the experiment with the selection lines showing that body mass is a determinant of the observed pattern. Studies done with parasitic insects that exhibit scramble competition consistently show that high density and small host size have deleterious effects on survivorship and body mass (Colegrave 1995) and thus impose selection on insects and influence the evolution of body size (Pexton and Mayhew 2004). Thus, the differential responses of beetles of different sized-populations/lines to seed size (significant population/lines-by-seed size interaction) and to competition I found (significant population/lines-by-larval density interaction) are possibly due to body size adaptation to seed size and to larval density (Messina 2004). Thus, I demonstrate that the magnitude of response depends on body size and that populations adapted to different sized hosts, and in consequence having different body size, may suffer in a different manner the consequences of competition.

Another interesting result from this study is that body size may impose a constraint on how large or how small beetles can be. The small *ANAPOIMA* beetles adapted to small seeds, though increasing significantly body size when developing at low densities and on larger seeds may have a reduced ability to increase body size on larger hosts. On the other hand, the larger *ORACLE* and *DEL RIO* beetles do not reach a significantly smaller size after being raised at 4 and 6 larvae per seed, and their survivorships decrease sharply, suggesting that some factor other than seed size is limiting their ability to exploit smaller hosts.

Concerning development time, previous studies have shown that, as host size decreases, development time decreases (Boivin and Lagace 1999, Mackauer and Chau 2001). I found a different result. When beetles were reared on the smallest seeds, *P. guachapele,* development time at all larval densities increased by ~ 5 days, and survivorship decreased by ~ 20%. In addition, there was not a significant difference of development on large and medium size seeds. As I suggested in chapter two, the smaller *P. guachapele* seeds are a poor nutritional food source and, as a consequence, beetles developing in those seeds either must feed more (extending development time) or assimilate resources more slowly than beetles feeding on *A. greggii*. Compensatory feeding has been found in numerous groups of organisms as a strategy to reach a target body size despite the deleterious effects of competition (Schoohoven 1998). Lowered feeding rate in group feeding animals can be caused by, among others, reduced food availability or by physical interaction among competitors (Gauvin and Giraldiu 2003).

Regarding the second prediction on whether the effects of larval competition vary with host size, with the deleterious effects being greatest on smaller hosts, the significant interaction between seed size x larval density for body mass confirm that larval competition varied among seed sizes, but the greatest reduction in body mass with increasing larval density occurred in medium seeds instead of smaller seeds. This result, in addition to the fact that *DEL RIO* beetles were the most affected by both seed size and larval density (significant population x larval density x seed size effect), suggest differences in competitive ability of different sized beetles as a result of their differences in body size for being adapted to different sizes of seeds. In small seeds the probability of encounters among competitors sharing a seed will be higher as larval density and larval body mass increase. Thus, it would be expected that greater mortality will occur as a result of higher encounter rates among competitors. Messina (2004) showed that *Callosobruchus maculatus* that had evolved different competitive strategies under natural conditions (scramble versus contest competition) switched their type of competition in a natural selection experiment in which beetles were allowed to adapt to different sizes of seeds - contest type beetles evolved in beetles adapted to small seeds and scramble competition evolved in beetles adapted to large seeds. Theory regarding the evolution of gregarious development, suggests two possible ways by which this could have evolved: decreased larval mobility and decreased larval aggressiveness (Brouder and Boivin 2004). *S. limbatus* larvae usually feed in near the surface of the seed reducing the probability of encounter with other larvae. Dissection of seeds indicates that, when seeds are smaller and larval density increases, encounters among larvae are more frequent (personal observation), a pattern consistent with findings of other studies (Pexton and Mayhew 2004 and references there in). Although contest competition have not been detected in *S. limbatus*, the non-linear effects of competition on survivorship and the lack of a difference in body mass above 4 larvae per seed on larger beetles and in smaller seeds are suggestive. Examination of interactions among larvae within small seeds could be enlightening to explaining the survivorship and size patterns observed here.

Finally, selection experiments are a powerful way to conduct this type of studies. An advantage of using this approach is that the genetic constitution of selected lines is originated from a single population reducing the confounding effects caused by adaptation to other factors such as host, temperature, latitude, natural enemies, etc, factors that indeed may affect the outcome when analyzing variation in natural populations. The two independent experiments show that, in most cases, similar responses of large versus small beetles to larval density and seed size were found using the selected and the natural populations. The only major difference was the significant population effect for development time when considering only all densities or densities above 6 larvae per seed in the natural populations. In the selected lines these effects were significant at all levels of analysis. This difference may be due that in the selection lines, the only factor affecting the observed response was body size meanwhile natural populations differed in a series of traits (see chapter two) that could affect the response at lower densities. The experiment with the body size selected lines allowed me to demonstrate that variation among populations in the effects of larval competition and seed size on larval growth and survival are partially a result of differences in body size and that these factors have been and are important determinants in the evolution of body size and other fitness traits in organisms with similar characteristics to *S. limbatus*.

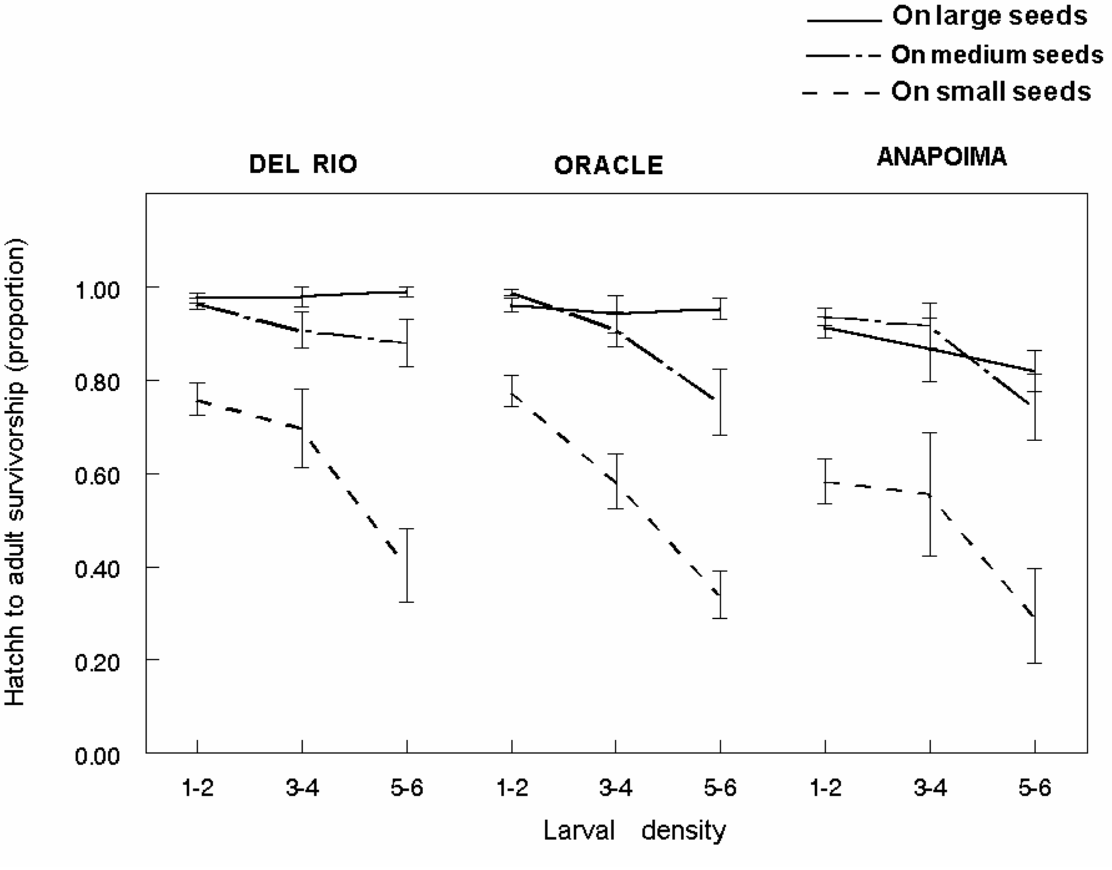

Figure 4.1: Hatch to adult survivorship on three populations of *S. limbatus* on three different seed sizes and at different larval densities.

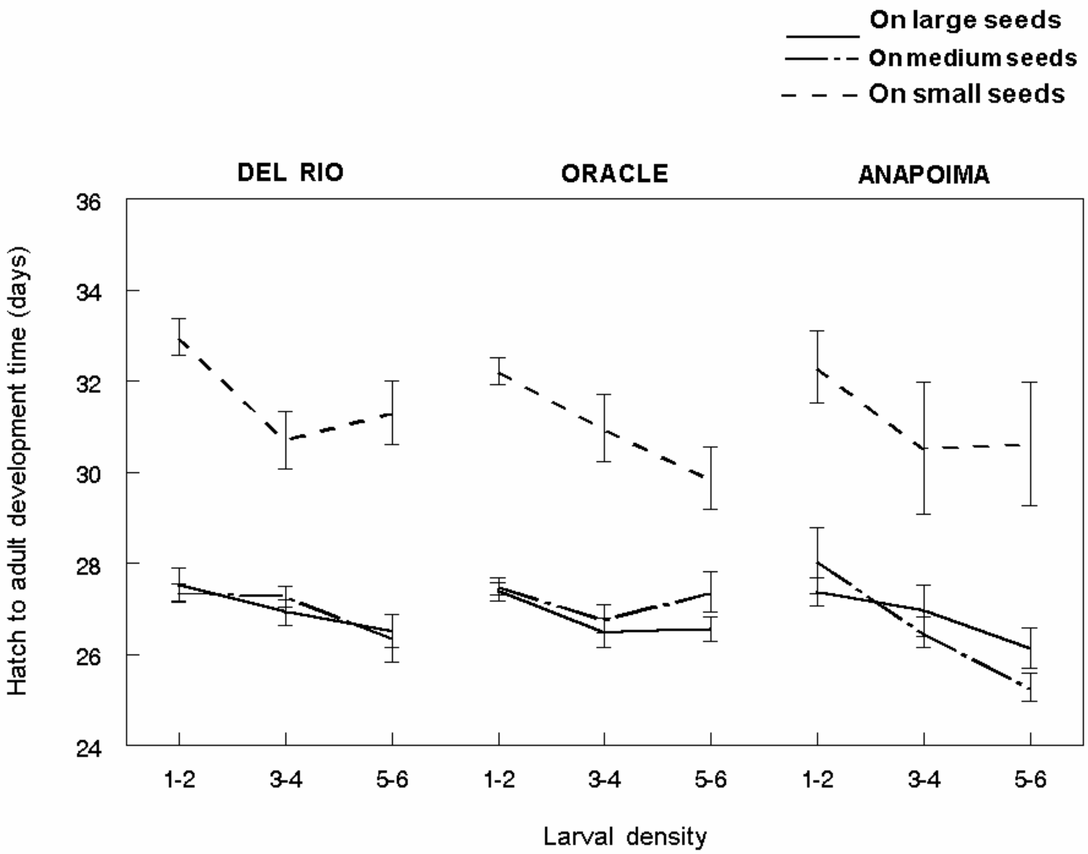

Figure 4.2: Hatch to adult development time of three populations of *S. limbatus* on three different seed sizes and at different larval densities.

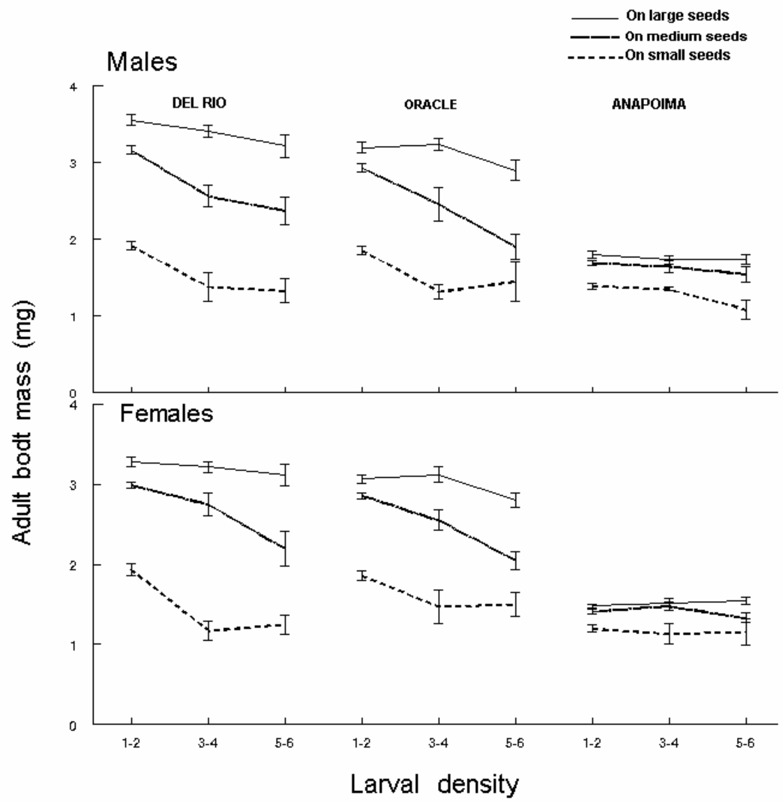

Figure 4.3: Emergence body mass of beetles from three populations of *S. limbatus* on three different seed sizes and at different larval densities.

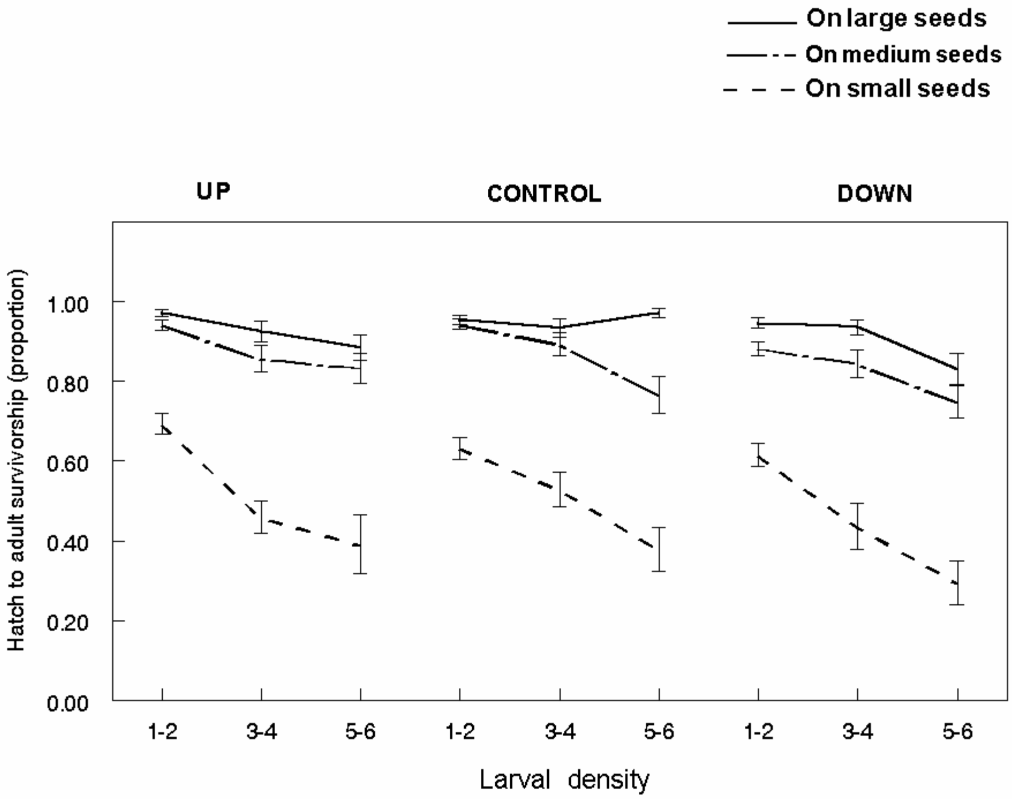

Figure 4.4: Hatch to adult survivorship on body size selected lines of *S. limbatus* on three different seed sizes and at different larval densities.

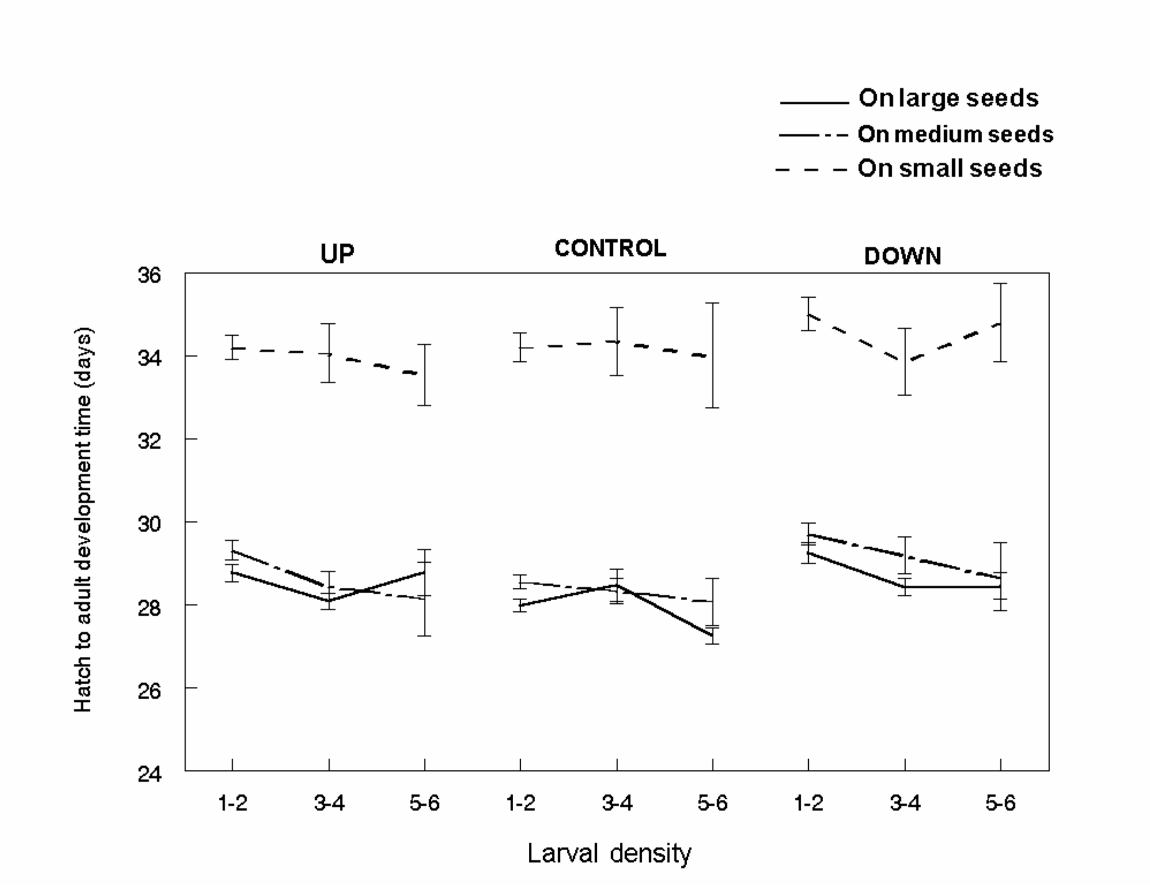

Figure 4.5: Hatch to adult development time of three body size selected lines of *S. limbatus* on three different seed sizes and at different larval densities.

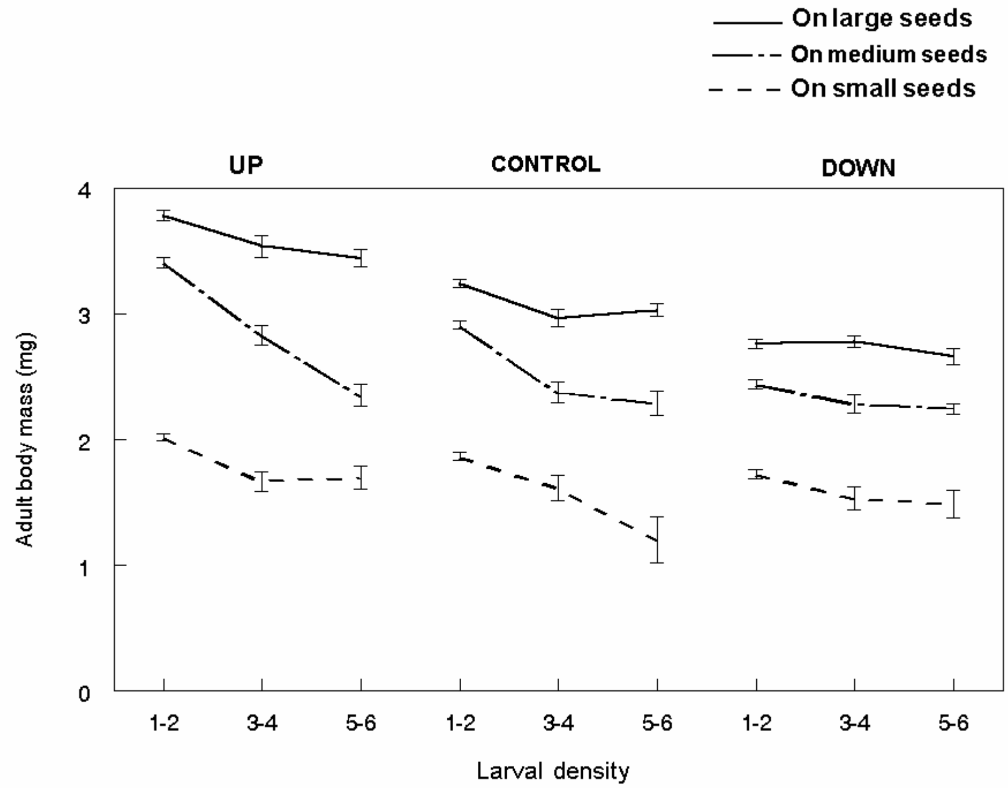

Figure 4.6: Emergence body mass of beetles from three body size selected lines of *S. limbatus* on three different seed sizes and at different larval densities.

Chapter 5: Conclusions, future directions and implications

Stator limbatus is a generalist seed parasite that feeds on seeds of approximately 80 host plants throughout its broad geographic distribution (Johnson and Kingsolver 1976, Johnson et al. 1989). However, populations have only a few hosts available at each locality. The populations studied here are from extreme ends of the geographic distribution of *S. limbatus* – Arizona and Texas in the United States and from Cundinamarca and Tolima in Colombia. The hosts used by these populations are non-overlapping – beetles in the United States use seeds of *Acacia greggii*, *Parkinsonia florida* and *Acacia berlandierii* (among others), all of which are large-seeded hosts, whereas the populations from Colombia use seeds of *Pseudosamanea guachapele*, which produces small seeds. Seeds of these species also vary substantially in their quality for beetle growth and development (Chapter two).

5.1 Local adaptation phenotypic plasticity and maternal effects

Populations used in this study represent very divergent clades in the phylogeography of the species (Morse and Farrell 2005a). Thus, I expected that populations that use different hosts, at different localities, would be adapted to seeds of their local hosts and that local adaptation and phylogenetic divergence caused a reduced ability to use alternative hosts (Van Zandt and Mopper 1998); i.e., that populations would have lower performance and survivorship on alternative hosts. Common garden experiments presented in chapter two, using Colombia and United States populations, revealed that populations are locally adapted to their native hosts. Local adaptation is based in genetic differences among Colombia and Arizona populations, and expressed in significant differences in age at first reproduction (Figure 2.2), number of eggs laid in the first 24 h. of oviposition (Figure 2.4), body mass at maturation (Figure 2.6), and larval survivorship (Figure 2.7). These results also show that adaptation to seed size and quality has strong influence in body size. Beetles adapted to small seeds of *P. guachapele* are smaller than beetles adapted to the large seeds of *A. greggii.*

These experiments also show that local adaptation does not restrict the use of alternative hosts in those populations, particularly in the Colombian populations in which performance was better in *A. greggii*, the host of the Arizona populations. Phenotypic plasticity, especially egg size plasticity, maternal effects and seed size and quality are factors that shape this pattern of response. Beetles developing in large seeds reach larger body size (Figure 2.6), have higher survivorship (Figure 2.7) and lay smaller eggs (Figure 2.3) than when developing in the small seeds of *P. guachapele*, responses that agreed with the findings of other studies comparing insects that use large vs. small hosts (Chapter two). However, contrary to expectations, development time was longest in small seeds, suggesting that *P. guachapele* seeds, in addition to be smaller, are of a lower nutritional value compared to seeds of *A. greggii*.

Though beetles from all populations experienced a similar pattern of response to host size, the magnitude of the responses varied. When beetles from Colombia were reared on the small *P. guachapele* seeds, body mass was 11.0% (females) and 16.5% (males) smaller than when beetles were reared on the larger seeds of *A. greggii*, whereas Arizona beetles were 29.5% smaller (females) and 24.5% (males). This result also show that in the Colombia populations the males experienced the highest reduction in body mass, while in the Arizona populations females were most affected (Figure 2.6).

Beetles whose mothers were reared on *P. guachapele* emerged about one day sooner than beetles whose maternal host was *A. greggii* regardless of the host on which progeny were reared (Figure 2.5). In addition, females exhibited egg size plasticity in response to their oviposition host. Irrespective of population of origin, females laid larger eggs on seeds of *P. guachapele* than on *A. greggii* (Figure 2.3). Despite the regular result that variation in egg size mediates variation in development time, the maternal rearing host effect on development time obtained in chapter two is apparently not due to changes in egg size; egg size was not affected by maternal rearing host and the maternal host effect on development time was still statistically highly significant after including egg size as a covariate in the statistical model. The observed maternal rearing host effect is thus more likely due to changes in egg composition, such as egg energy reserves, maternally produced proteins (such as regulatory proteins or enzymes), or maternal mRNAs (Chapter two). The examination on how maternal effects influence the composition of eggs in arthropods other than *Drosophila* is needed. Fox et al. (1995) also found that maternal rearing host affected offspring body size (offspring were larger when their mothers were raised on *P. florida*), but no such effect was found in this current study. Neither Fox et al. (1995) nor this current study found any evidence that offspring have higher fitness (higher survivorship, reduced development time or larger body size) when raised on the same host as their mother (i.e., no significant maternal host x offspring host interactions).

This the first time egg size plasticity is demonstrated in populations of *S. limbatus* in response to a seed trait different than toxicity of the seed coat (Chapter three, experiment four). This study is also the first to demonstrate that egg size plasticity is present in populations of *S. limbatus* other than the Arizona populations. Because egg size plasticity is present in populations with a large phylogenetic divergence, it is likely an ancestral trait that allowed (and continues to facilitate) diet expansion via colonization of new hosts (Chapters two and three).

5.2 Host discrimination and oviposition behavior in *S. limbatus*

Given that local adaptation does not limit the use of alternative hosts in these populations, and that performance of beetles was greater in the larger size and higher quality seeds of *A. greggii*, I analyzed female oviposition behavior and host discrimination to quantify the influence of female body size, seed size and seed quality on the preference of females for a given host. I found that females minimize the deleterious effects of small seed size, low seed quality and the increased larval density caused by superparasitism. Specifically, females laid more eggs (Figure 3.1; Chapter three, experiment one) and distributed eggs more evenly among seeds (Figure 3.2; chapter three, experiment one) on non-parasitized seeds than on seeds already parasitized. Females also preferred larger seeds over smaller seeds, (Figures 3.3 and

3.4; chapter three, experiment two) and preferentially laid eggs on higher quality *A. greggii* than on lower quality *P. guachapele* seeds (Figures 3.6 and 3.7; chapter three).

Thus, host discrimination and oviposition preference experiments showed that size and quality of seeds are mayor determinants of host preference, and that local adaptation does not restricts the possibility of recognizing and using alternative hosts.

5.3 Effects of seed size and insect size in the consequences of larval competition

Once eggs are laid, one of the major factors affecting life history traits is competition among siblings. In chapter four I presented two experiments developed to determine how beetles differing in body size, and from populations adapted to different host species, respond to variation in larval competition on large versus small seeds and on high quality versus low quality seeds. To disentangle effects of body size from population differentiation in other traits I performed the experiment twice, once using beetles from populations naturally differing in body size and once using laboratory selected lines differing in body mass but created by artificial selection from a single Arizona population. I observed significant density dependence in all study populations – beetles reared at high density, and on small seeds, were smaller and had reduced survivorship (Figures 4.1 and 4.3; chapter four). However, populations responded differently to larval competition and seed size in a manner largely consistent with the hypothesis that large-bodied beetles suffer greater fitness consequences of high density and small seed size – the larger Del Rio beetles were more affected than the smaller Anapoima beetles. Larval competition also varied among seed sizes, but the greatest reduction in body mass with increasing larval density occurred in medium seeds instead of smaller seeds (Figure 4.3; chapter four). This, in addition to the fact that Del Rio beetles were the most affected by both seed size and larval density (significant population x larval density x seed size effect), suggest differences in competitive ability of different sized beetles as a result of their differences in body size.

5.4 Future directions

5.4.1 Influence of natural enemies in host plant use

Several papers have addressed the importance of natural enemies in shaping the evolution of insect-plant interactions (Bernays and Graham 1988, Hawkins and Lawton 1987), having among other, important consequences for the evolution of specialization (Nosil et al. 2002). In some cases, parasitism is strongly host-plant dependent with some parasitoid species specialized with respect to the host found in specific plant species (Lill et al. 2002). In other, herbivores may escape from natural enemies being scare in space and/or, or chemically defended by metabolizing plant allelochemicals (Ballabeni et al. 2001, Stamp 2001). It has been also suggested that host use is also determined by enemy free space and that in addition, and sometimes in opposition, to organisms using and preferring larger and better hosts for offspring development, females select less suitable hosts for offspring development, but with a lower risk of mortality by natural enemies. Preliminary studies on the effects of natural enemies for the populations here examined are currently undergoing. Seeds from a minimum of twenty trees from each host and from each population were collected and seeds bearing eggs were split in single petri dishes and placed in a growth chamber at 28^{o}C. The number of *S. limbatus* emerging adults, parasitoids and predators emerging from those seeds were collected and scored. They will be classified in relation with *S. limbatus* as predator or parasitoid. Also, the number of eggs laid on each seed, its distribution, and the proportion of hatched versus unhatched eggs will be recorded. This will give an indication of the relative amount of pressure imposed by natural enemies on each host and how it varies among hosts.

5.4.2 Allocation strategies in populations of *S. limbatus*

The rearing procedure use in this research for the Colombia and the United States populations show differences in the mating strategies and food supply. Colombia beetles require adult food sources to produce eggs (they are income breeders), while

The United States beetles do not (they are capital breeders). Such variation in allocation strategies within a species provides an exciting opportunity to study the factors that could mediate the evolution of allocation strategies. A possible explanation for this difference is that selection on populations from each clade affects differentially the life cycle of beetles. Populations in Colombia have a continuous supply of food source in the form of nectar and water, while the United States populations do not. In this way, Colombia beetles that develop in small, low quality seeds and in consequence are smaller, would require extra food supply to make eggs under the presumption that larval development in these beetles is primarily directed to survive in a low quality host. On the other hand, The United States beetles do not have sources of food for adults during most of the year given the extreme deserts in which hosts plants inhabit; in addition, compared with the Colombian hosts, seed hosts for these populations provide good quality nutrients for development. Thus, it is expected that larval development will generate beetles that once emerge start laying eggs sooner as was found in this research.

5.5 Implications for host colonization and diet expansion.

Knowledge of how variation in host plant quality and host size influence variation in life histories during development, and how genetic differentiation affects the responses of populations to these plant factors, are essential in the understanding of host colonization and diet expansion of herbivorous insects. This dissertation shows that variation in life history traits due to the factors mentioned above are highly influenced by differences in body size, maternal effects and the amount of plasticity, effects that are of special importance when considering the evolution of diet breadth in organisms that like *S. limbatus* experience resource limitation during development (i.e. parasitoids and seed feeders).

I have also demonstrated that plasticity in life history traits and maternal effects facilitate responses of organisms to alternate plants which differ in size and quality. Thus, the environment mothers experience allow them to set up specific changes in the offspring (i. e. increase egg size, that produce larger body sized progeny) that may increase their fitness when using new introduced hosts, and prepare them to survive and exploit a new environment. For example, in the case of insects that are pests of agricultural crops, plasticity may favor the colonization of new crops without causing changes in the genotype of the populations when exposed to the new environment. However, because the same genotype expresses different phenotypes in each environment, plasticity may also buffer herbivores from selection post colonization. In other words, genetic differentiation and attainment of an optimal phenotype on each plant would be slower if gene flow is maintained among organisms using the two sources (the native and the novel hosts), and thus local adaptation to host plants would be unlikely.

In addition, populations adapted to different size and different quality hosts show significant differences in plasticity when exposed to novel hosts, and as result, the outcome of using alternate hosts under stressful conditions such as competition and decreased host size is influenced by the size of organisms belonging to each population. This is also an important aspect to consider when making generalizations about the responses of species to host plants based on one or a few populations studied. This dissertation demonstrated that a species with a broad distribution in which populations have only a few hosts available respond differentially to changes in host size, quality and under intraspecific competition.

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EDUCATION

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B.S.	Education in Biology. December 1990. Department of Biology. Universidad Pedagógica Nacional. Bogotá, Colombia.

PROFESSIONAL POSITIONS

2006-present.	Assistant Professor. Facultad de Estudios Ambientales y Rurales. Pontificia Universidad Javeriana. Bogotá, Colombia.
2001-2005.	Research Assitant. Department of Entomology. University of Kentucky. USA.
1994-1997.	Professor. Universidad de Los Andes and Universidad Nacional de Colombia. Bogotá, Colombia.

AWARDS AND HONORS

- Academic Scholarship, Department of Entomology, University of Kentucky, 2001
- Research Assistantship. Department of Entomology, University of Kentucky. 2001-2005.

- Second prize winner. Meeting Entomological Society of America. Nov. 2003. Section Cd: Ecology and Behavior.

- Third place: Annual Photo Contest. XXIII Congreso de la Sociedad Colombiana de Entomología. Cartagena. Julio 17-19 de 1996.
- Honorary Scholarship. Program Masters in Biology. Universidad Nacional de Colombia. Second semester of 1995.
- Honorary Scholarship. First semester of 1995. Program Masters in Biology. Universidad Nacional de Colombia.
- Honorary mention in the First Annual Photo Contest. Association for Tropical Lepidoptera. 1992.
- Honorary Scholarship.1987. Universidad Pedagógica Nacional.

PUBLICATIONS

Book chapters

AMARILLO, A. 2002 Sistemática de animales venenosos. En: Pineda D. (ed). Accidentes por animales venenosos. Instituto Nacional de Salud.

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