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Ecology and Evolution of Diet Expansions to Exotic Hosts in Generalist and Specialist Rolled Leaf Beetles (Genus Cephaloleia, Coleoptera; Chrysomelidae)

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UNIVERSITY OF MIAMI

ECOLOGY AND EVOLUTION OF DIET EXPANSIONS TO EXOTIC HOSTS IN GENERALIST AND SPECIALIST ROLLED LEAF BEETLES (GENUS *CEPHALOLEIA,* COLEOPTERA; CHRYSOMELIDAE)

By

Carlos García-Robledo

A DISSERTATION

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of the University of Miami
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The interactions between plants and their insect herbivores are one of the main generators of biological diversity. A fundamental process generating this outstanding diversity is diet expansion to novel host plants. During the last four decades scientists accumulated evidence showing that co-adaptation between plants and herbivores is a major process assembling plant-herbivore interactions. However, rescent research suggests that adaptation is not always a prerequisite to generate novel plant-herbivore interactions. Novel associations between plants and insect herbivores may be assembled by ecological fitting – an ecological process whereby herbivores colonize novel host plants as a result of the suites of preadapted traits that they carry at the time of colonization.

A widespread assumption concerning the architecture of insect herbivore genotypes is the “Jack of all trades master of none” principle. This principle proposes that there is a trade-off in genotype performances between host plants. The main prediction of this principle is that genotype performance will be negatively correlated among hosts. Genotypes displaying high performance on a given host will perform poorly on other hosts. This constraint of adaptation to multiple host plants implies that diet specialization will be selected over generalization. Contrary to these theoretical expectations, in most cases, genotypes that perform well in one host will also perform well in other host plants.

Positive correlations in cross-host performance represent ecological and evolutionary dynamics opposite to the “Jack of all trades” principle. In this scenario genotypes with high performance on one host plant also have high performance on other plants, promoting generalization.

The predictions of the current theory on the assemblage of novel plant-herbivore interactions focus on the fact that most insect herbivores are specialists. However, to fully understand the processes underlying the assembly of novel plant-insect interactions, it is necessary to study diet expansions in both specialist and generalist insect herbivores.

This dissertation was performed at La Selva Biological Station, a tropical rain forest in Costa Rica, Central America. We studied a group of neotropical herbivores, the “rolled-leaf beetles” (*Cephaloleia,* Chrysomelidae: Cassidinae) and their host plants, neotropical plants in the order Zingiberales. *Cephaloleia* beetles have evolved with neotropical Zingiberales for the last 40-60 MY. Four paleotropical and one South American members of the Zingiberales have been introduced to La Selva during the last decade. After these introductions, currently seven *Cephaloleia* beetles are expanding their diets to exotic Zingiberales. These incipient diet expansions represent an opportunity to understand: 1) the relative roles of adaptation vs ecological fitting on the demography and colonization success of novel hosts in generalist and specialist herbivores and 2) whether genotypic performance across original and novel hosts are negatively correlated, as predicted by the “Jack of all trades” principle, or genotype performances across original and novel hosts are positively correlated.

For most of the experiments included in this dissertation, I focused on the performance of larvae and adults of two generalist (*Cephaloleia belti, C. dilaticollis*) and two specialist beetles (*Cephaloleia dorsalis*, *C. placida*) reared in the laboratory on native or exotic Zingiberales. Generalist and specialist species display similar responses when changing their diets to novel hosts. Larvae preferred and performed better in the original than in the novel hosts. Adults usually displayed the opposite pattern, *i.e.* higher preference for and longevity on the exotic than on the novel hosts. In most novel interactions between *Cephaloleia* beetles and exotic Zingiberales, larval performance required adaptation, but adult performance was pre-adapted to the novel hosts. Therefore, both adaptation and ecological fitting are playing a role during diet expansions to novel hosts.

Vital rates estimated through experimental demography show that population growth is reduced on novel host plants for both generalist and specialist *Cephaloleia*. Although in some cases population growth on the novel hosts is negative, suggesting the potential outcome of extinction after colonization or source-sink dynamics, several beetle species displayed positive population growth in the novel host plants. Positive instantaneous population growth rates in novel hosts supports diet expansions without substantial initial evolutionary change through ecological fitting.

In quantitative genetics experiments testing for cross-host genetic correlations in performance between the original and novel host plants, we did not find evidence for negative genetic correlations, as predicted by the “Jack of all trades” principle. Most genetic correlations in performance between original and novel hosts were either not significant or they were positive. These results represent very different ecological and evolutionary dynamics than those predicted by the “Jack of all trades” principle. In this case, genotypes with high performance on original hosts also displayed high performance on novel hosts, promoting generalization. In conclusion, interactions between *Cephaloleia* beetles and plants from the order Zingiberales are labile. In some cases diet expansions may occur without substantial evolutionary change. In addition the genetic architecture of genotypes promotes generalization during diet expansions to novel hosts.

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I want to thank my committee members, Carol C. Horvitz, Theodore H. Fleming, Douglas J. Futuyma, Keith D. Waddington and Barbara A. Whitlock. Charles L. Staines (Smithsonian Institution) shared his expertise on *Cephaloleia* beetles and provided invaluable advice during the different phases of this project. I thank E.K. Kuprewicz for her advice during the design of experiments, proposal writing, fieldwork and for making our time at La Selva and beyond so fun. I thank the staff of La Selva Biological Station - Organization for Tropical Studies, specially Deedra McClearn , Orlando Vargas, Danilo Brenes, Bernal Matarrita Beto Garcia and Mauricio Gaitán Herrera. I thank G. Frias, J.G. Huertas, G. Huertas and R. Sanchez, for assistance in the field and laboratory. SEM images were provided by P. Blackwelder, H. Al Sayegh, and X. Vial - University of Miami Center for Advanced Microscopy (UMCAM). The illustrations included in this dissertation were made by E. K. Kuprewicz. This research was funded by the J. McLamore Fellowship - University of Miami and the OTS (Organization for Tropical Studies)—Donald and Beverly Stone Fellowship and the OTS-Christiane and Christopher Tyson Fellowship to C. García-Robledo and the Cooper Fellowship, College of Arts and Sciences, University of Miami to C. C. Horvitz. Comments on one or multiple chapters by C. C. Horvitz, T. H. Fleming, D. J. Futuyma, J. Gómez-Zurita, P. Jolivet, E. K. Kuprewicz, C. C. Labandeira, J. Prena, C. L. Staines, D. R. Strong, K. D. Waddington and B. A. Whitlock improved this dissertation substantially.

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Chapter I

Introduction: Diet expansion to novel hosts in a group of Neotropical insect herbivores

The interactions between plants and their insect herbivores are one of the main generators of biological diversity (Mayhew 2001). A fundamental process generating this outstanding diversity is diet expansion to novel host plants in ecological time (Agrawal 2007, Janz et al. 2006). During the last four decades, scientists accumulated evidence showing that co-adaptation between plants and herbivores is a major process assembling plant-herbivore interactions (Ehrlich and Raven 1964, Janzen 1980, Ueno *et al.* 2003, Futuyma 2008). However, adaptation is not always a prerequisite to generate novel plant-herbivore interactions (Agosta 2006, Agosta and Klemens 2008, Agosta and Klemens 2009). If the traits required for obtaining realized fitness are pre-adapted to the characteristics of the novel hosts, novel plant-herbivore interactions may be assembled by ecological fitting – an ecological process whereby herbivores colonize novel host plants as a result of the suites of preadapted traits that they carry at the time of colonization (Agosta 2006, Agosta and Klemens 2008, Agosta and Klemens 2009).

The success or failure of novel plant-herbivore interactions depends on the genetic variation and genetic architecture of genotypes of herbivore populations. The presence of genetic variation within the population for the traits involved in host colonization will increase the probability for a genotype to be able to persist in the novel host plant. A widespread assumption concerning the architecture of insect herbivore genotypes is the "Jack of all trades master of none" principle (McArthur 1972, Futuyma and Moreno 1988, Via 1990, Futuyma et al. 1995). This principle proposes that there is a trade-off in genotype performances between host plants. The main prediction of this principle is that genotype performance will be negatively correlated among hosts (McArthur 1972, Futuyma and Moreno 1988, Via 1990, Futuyma et al. 1995). Genotypes displaying high performance on a given host will perform poorly on other hosts. This constraint of adaptation to multiple host plants implies that diet specialization will be selected over generalization. Contrary to these theoretical expectations, most empirical evidence supports positive correlations for genotypes on different host plants (Futuyma and Philippi 1987, Ueno *et al.* 2003, Futuyma 2008). In this case, genotypes that perform well on one host will also perform well on other host plants. Positive correlations in cross-host performance represent very different ecological and evolutionary dynamics than those predicted by the "Jack of all trades master of none" principle. In this case, high performance on a given host plant enhances the herbivore's performance on other plants, promoting generalization.

The predictions of the current theory on the assemblage of novel plant-herbivore interactions focus on the fact that most insect herbivores are specialists (Fox and Morrow 1981, Jaenike 1990, Thompson 1995, Novotny and Basset 2005). However, insect herbivores can be also generalists (Singer 2001). Because insect herbivores with broad diets experience different environments and specialists do not, it is possible that the role of adaptation, or ecological fitting, and the architecture of insect herbivore genotypes depend on insect herbivore diet breadths.

This dissertation explores the roles of adaptation and ecological fitting on the demographic vital rates and colonization success of novel hosts in a group of generalist and specialist neotropical herbivores. Additionally, I explore the architecture of genotypes during incipient diet expansions to novel hosts, exploring whether correlations in performance (predicted by the “Jack of all trades” principle), are the rule.

One of the oldest and most conservative plant-herbivore associations is the interaction between neotropical gingers (Order Zingiberales) and beetles of the neotropical genus *Cephaloleia* (Chrysomelidae, Cassidinae) (Wilf et al. 2000; McKenna and Farrell 2005; McKenna and Farrell 2006; García-Robledo and Staines 2008). *Cephaloleia* beetles are also known as the “rolled-leaf beetles” because the adults of most species feed and mate inside the scrolls formed by the young rolled leaves of their host plants (Strong 1977). It is estimated that *Cephaloleia* beetles and neotropical Zingiberales have interacted for the last 40 – 60 MY in isolation from paleotropical Zingiberales (Wilf et al. 2000; McKenna and Farrell 2006; García-Robledo and Staines 2008).

I conducted this research at La Selva Biological Station (hereafter La Selva) from August 2005 to March 2009. La Selva is a tropical rain forest in Costa Rica, Central America (10º26’N, 83º59’W) (Figure 1.1). At La Selva, at least 40 species of *Cephaloleia* associate with at least 43 native plants from the order Zingiberales (Staines 1996). In the last decade, four paleotropical and one South American species of exotic Zingiberales have been found at La Selva (Figure 1.2, Tables 1.1; *see* origin of exotic Zingiberales in Table 5.1). An additional exotic plant from eastern Asia, *Apinia zerumbet* (Zingiberaceae) was rescently reported near the boundary of the La Selva property (Figure 1.2, Table 1.1). Seven *Cephaloleia* species with contrasting diet breadths are currently expanding their diets to the exotic Zingiberales, generating 17 novel plant-herbivore interactions (Figure 1.2, Table 1.1, pictures of *Cephaloleia* species in Appendix 1).

These incipient diet expansions to exotic Zingiberales by *Cephaloleia* beetles present an opportunity to study the processes involved in the establishment of novel plant-herbivore interactions. In the initial chapters of this dissertation (Chapters 2 to 4), I focused on the natural history of *Cephaloleia* beetles on their original host plants. In Chapter 2, I re-analyzed the fossil evidence describing this plant-herbivore association as one of the oldest and most conservative plant-herbivore interactions. Chapter 3 is a detailed description of the natural history, larval morphology and adult longevity of two generalist (*Cephaloleia belti* and *C. dilaticollis*) and two specialist (*Cephaloleia dorsalis* and *C. placida*) insect herbivores on their original host plants. These four beetle species are the focus of further chapters exploring the evolutionary processes involved in diet expansions, demography and genetics of incipient plant-herbivore interactions. In Chapter 4, I explored the role of scents from native host plants in the attraction of *Cephaloleia* insect herbivores.

The following three chapters (Chapter 5-7) are the core of this dissertation. In Chapter 5, I explored the relative roles of adaptation and ecological fitting in the establishment of novel plant-herbivore interactions in generalist and specialist herbivores. I determined the role of these processes on *Cephaloleia* larval and adult preference and survival in novel host plants.

In Chapter 6, using an experimental demography approach, I estimated the vital rates of generalist and specialist *Cephaloleia* beetles when reared on original or novel host leaf tissue. Vital rates were incorporated into a cohort life-table to estimate fundamental demography parameters such as the instantaneous population growth rate on original and novel hosts.

Finally, in Chapter 7, I explored the genetic architecture of *Cephaloleia* genotypes expanding their diets to novel hosts. Using quantitative genetic experiments, I determined if genetic correlations give support to the “Jack of all trades” principle, or alternative patterns such as not significant or positive genetic correlations.

Table 1.1. *Cephaloleia* species expanding their diets from original to novel host plants. Diet records from a. specimens at the NMNH entomology collection, Smithsonian Institution, data from C.L. Staines and ca. 2500 individual host plants surveyed by C. García-Robledo from August 2005 to March 2009.

Beetle species	Original hosts	Novel hosts
Cephaloleia belti Baly	**Heliconiaceae** *Heliconia imbricata, H. irrasa, H. latispatha, H. mariae, H. mathiasiae, H. pogonantha, H. wagneriana.* **Marantaceae** *Calathea cleistantha, C. crotalifera, C. hammelii, C. inocephala, C. lutea, C. marantifolia, Pleiostachya pruinosa.* **Cannaceae** *Canna tuerckheimii*	**Heliconiaceae** *Heliconia psittacorum.* **Musaceae** *Musa velutina* **Costaceae** [1]*Cheilocostus speciosus.*
Cephaloleia congener Baly	**Heliconiaceae** *Heliconia imbricata, H. irrasa, H. latispatha, H. mathiasiae, H. pogonantha, H. tortuosa., H. wagneriana* **Marantaceae** *Calathea cleistantha, C. crotalifera, C. gymnocarpa, C. inocephala, Ischnosiphon inflatus*	**Heliconiaceae** *Heliconia psittacorum* **Musaceae** *Musa velutina*
Cephaloleia dilaticollis Baly	**Heliconiaceae** *Heliconia latispatha, H. mariae.* **Marantaceae** *Calathea crotalifera, C. inocephala, C. lasiostachia, C. lutea, C. marantifolia, C. similis,* **Zingiberaceae** *Renealmia alpinia, R. cernua*	**Musaceae** [1]*Musa velutina.* **Zingiberaceae** *Hedychium coronarium, Alpinia purpurata, A. zerumbec.*
Cephaloleia dorsalis Baly	**Costaceae** *Costus bracteatus, Costus laevis, Costus malortieanus, Costus pulverulentus.* **Marantaceae** [1]*Calathea leucostachys.*	**Costaceae** *Cheilocostus speciosus.*
Cephaloleia placida Baly	**Zingiberaceae** *Renealmia alpinia, Renealmia cernua*	**Zingiberaceae** *Hedychium coronarium, Alpinia purpurata.*

Table 1.1 (Contd.)

Beetle species	Original hosts	Novel hosts
Cephaloleia reventazonica Uhmann	**Heliconiaceae** *Heliconia latispatha.* **Marantaceae** *Calathea crotalifera, C. inocephala, C. lutea, C. marantifolia.* **Cannaceae** *Canna tuerckheimii*	**Heliconiaceae** *H. psittacorum.* **Musaceae** *Musa velutina.* **Zingiberaceae** *Hedychium coronarium.*
Cephaloleia trimaculata Baly	**Zingiberaceae** *Renealmia sp.*	**Zingiberaceae** [1]*Hedychium coronarium,* [1]*Alpinia purpurata.*

[1] Rare events. Only one to four individual recorded during the surveys of ca. 2500 individual host plants from August 2005 to March 2009.

Figure 1.1. Study site. La Selva Biological Station, Costa Rica, Central America.

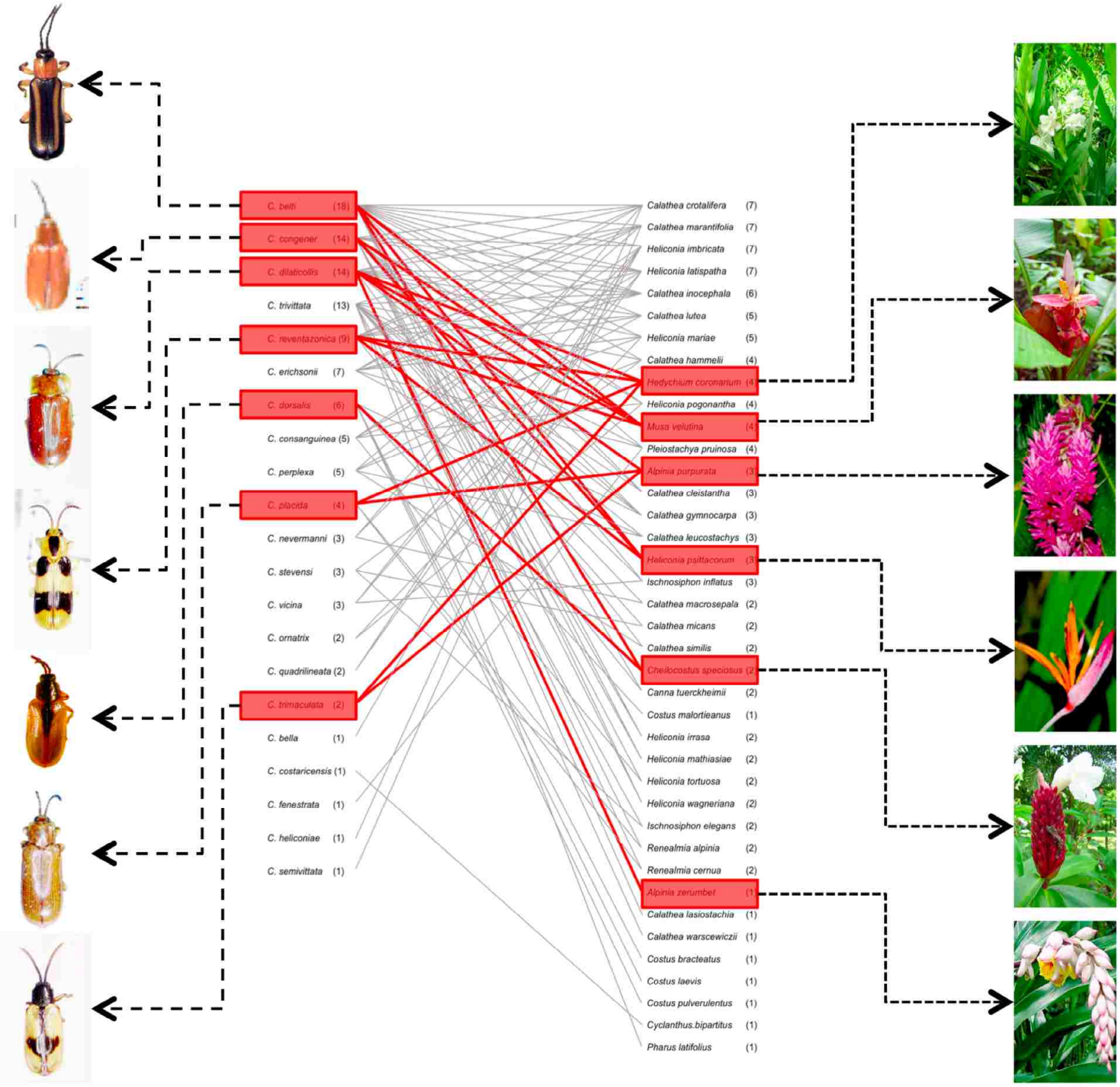

Figure 1.2. Diagram of known interactions between *Cephaloleia* beetles (left column) and their host plants (right column). The number in parethesis next to each beetle species represents the number of host plants for each herbivore. The number in parethesis next to each plant species represents the number of beetle species feeding on each host plant. The Gray lines represent interactions with native host plants. Red lines represent interactions with exotic Zingiberales reported at La Selva Biological Station in the last decade. Beetle species in red represent beetles currently expanding their diets to novel hosts. Host plants in red represent exotic hosts at La Selva Biological Station. Note two non-Zingiberales host plants: *Cyclanthus bipartitus* (Cyclanthaceae) and *Pharus latifolius* (Poaceae). Diet records from a. records from specimens at the NMNH entomology collection, data from C.L. Staines and ca. 2500

Chapter II

Herbivory in gingers from latest Cretaceous to present: is the ichnogenus *Cephaloleichnites* (hispinae, coleoptera) a rolled-leaf beetle?[1]

SUMMARY

It is suggested that rolled-leaf hispine beetles (Hispinae, Coleoptera) and plants from the order Zingiberales maintained a highly specialized plant-herbivore interaction for > 60 My. The evidence supporting this old and conservative interaction are herbivory marks found on leaves of the genus *Zingiberopsis* (Zingiberaceae) from the latest Cretaceous and early Eocene. This fossil herbivory was described as the ichnotaxon *Cephaloleichnites strongii* (Hispinae, Coleoptera), based on the assumption that this type of herbivory can be solely attributed to extant rolled-leaf beetles. This ichnotaxon has been a key element in several analyses on the origin, radiation and diversification of tropical insect herbivores. In this paper we report feeding patterns equivalent to those described in *Zingiberopsis* fossils but produced by larvae of Pyralidae and Choreutidae (Lepidoptera) and *Anopsilus* weevils (Curculionidae, Coleoptera) in four families of extant Zingiberales. We discuss the implications of *C. strongii* not being a rolled leaf beetle and how this may affect the current knowledge of the co-diversification of rolled-leaf beetles and their host plants from the order Zingiberales.

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[1] García–Robledo, C. and C.L. Staines. (2008). Herbivory in gingers from latest Cretaceous to present: is the ichnogenus *Cephaloleichnites* (Hispinae, Coleoptera) a rolled-leaf beetle? Journal of Paleontology. 82: 1035 - 1037

BACKGROUND

In the paper *Timing the radiation of leaf-beetles: Hispines on gingers from latest Cretaceous to recent*, (Science, Vol 289, Jul 2000), Wilf et al., proposed that rolled-leaf hispine beetles (Hispinae, Coleoptera) and plants from the order Zingiberales maintained a highly specialized plant-herbivore interaction in the new world for > 60 My (Wilf et al., 2000, Chaboo, 2007). They concluded this based on feeding tracks present in the leaves of 11 fossil specimens of the genus *Zingiberopsis* (Zingiberaceae) from the latest Cretaceous and early Eocene (Hickey and Stevenson, [Peterson in references] 1978; Wilf et al., 2000). Wilf et al., assured that these herbivory marks can be solely attributed to rolled-leaf beetles based on its similarity with the herbivory patterns described for hispines feeding on extant *Heliconia* (Heliconiaceae) (Strong, 1977). Wilf et al. (2000) proposed the ichnotaxon *Cephaloleichnites strongii* (Hispinae, Coleoptera) for the fossil herbivory (Figures 2.1A, 2.1C, 2.1E, 2.1H).

Since the publication of the paper by Wilf et al., (2000), the ichospecies *Cephaloleichnites strongii* (Hispinae, Coleoptera) has been a key element in several analyses on the origin, radiation and diversification of tropical insect herbivores (McKenna and Farrell, 2006; Gómez-Zurita et al., 2007). In a study on the herbivore communities in extant Zingiberales from Central and South America, we found that the fossil herbivory formerly attributed only to hispine beetles can be also produced by other extant insect herbivores. Here we report feeding patterns equivalent to those described in *Zingiberopsis* fossils but produced by Lepidoptera and Curculionidae in four families of extant Zingiberales.

METHODS

This study was performed in April 2007 at La Selva Biological Station, a tropical rain forest in Costa Rica, Central America (N 10° 26’ W 84° 00’) and in March 2006 in South America, in a tropical montane forest in the Peruvian Andes, Municipio de Aguas Calientes, at 2400 m (S 13° 9’ W 72° 32'). Larvae of Lepidoptera feeding on expanded leaves of Zingiberales were collected at La Selva Biological Station (Table 2.1). Larvae were brought to the laboratory and individually fed 10x10 cm of expanded leaf from their host plant. Larvae from the family Pyralidae feed on leaves covered with leaf litter (McCoy 1984**.** Therefore, in the herbivory trials involving pyralids, leaves were offered covered with a piece of leaf litter collected from the top of the leaves of the host plant. The feeding patterns were recorded after 48 h.

In the Municipio de Aguas Calientes, Peru, we collected curculionid beetles feeding on young unexpanded leaves of *Canna bangii* Kraetzl 1912. (Cannaceae, Zingiberales) (Table 2.1). To determine the characteristics of the damage produced on the leaf blade by curculionids, we offered one 1.5 X 1.5 cm section of fresh *C. bangii* leaf tissue to each weevil. After 12 hours, we recorded the shape of the damage produced on each leaf section. Finally, we compared the herbivory patterns observed in the laboratory with the patterns produced by hispine and non-hispine herbivores in nature.

RESULTS

In a tropical rain forest in Costa Rica, early instars of Pyralidae and Choreutidae (Lepidoptera) feed on expanded leaves of Heliconiaceae, Marantaceae and Zingiberaceae (Figs. 2.1B, 2.1D, 2.1G, 2.1I). In a tropical montane forest in Peru, *Anopsilus* Kirsch 1869 weevils (Curculionidae) feed on young rolled leaves of *Canna bangii* (Cannaceae). In the laboratory, both lepidopterans and curculionids produced herbivory marks

equivalent to those described in *Zingiberopsis* (Fig. 2.1) (Wilf et al., 2000*)*. Lepidoptera and Curculionidae removed leaf-tissue in linear strips between parallel veins, leaving the epidermis intact. The damage may be bordered by dark reaction tissue and the terminations of the strips are asymmetrically rounded (Figs. 2.1B, 2.1D, 2.1F, 2.1G, 2.1I). The herbivory marks observed in the laboratory were equivalent to those produced in nature by *Anopsilus* weevils and first instar larvae of Pyralidae and Choreutidae .
The relative frequency of hispine vs. non-hispine damage was highly variable among the four species of Zingiberales included in this study. In *Canna bangii* (Cannaceae) no hispine beetles were recorded. Therefore all the herbivory observed in the field could be attributed to *Anopsilus* weevils. In *Calathea crotalifera* (Marantaceae) herbivory by both Choreutidae and Hispinae co-occurred in most of the leaves. In *Heliconia* (Heliconiaceae) and *Renealmia* (Zingiberaceae), most of the herbivory observed was produced by hispine beetles. Damage by pyralids was restricted to areas where the host leaves were covered by leaf litter.

DISCUSSION

Our results suggest that hispine beetles are not the only herbivores able to produce the herbivory pattern described by Wilf et al. (2000) in extant Zingiberales. A question that arises from our results is how probable is that the Pyralidae, Choreutidae and Curculionidae are the actual culprits of the herbivory damage in *Zingiberopsis* fossils.

Pyralidae and Choreutidae are both members of the Apoditrysia, a relatively derived clade of ditrysians. Pyraloids, with a fossil record extending to the early Eocene, belong to the diverse clade Obectomera (Kristensen and Skalski, 1999; C. C. Labandeira personal communication 2007). The family Pyralidae, however, is a more recent lineage, and extends perhaps to middle Eocene Baltic amber (~44.5 Mya); choreutids lack a fossil record but nevertheless are basal apoditrysians (Kristensen and Skalski, 1999; C. C. Labandeira personal communication 2007). Given this, it seems that both the Pyralidae and by phylogenetic relationship, the Choreutidae, could produce the hispine-like damage during the mid-Paleogene, around the early to middle Eocene, *i.e.* the more recent "hispine" damage in Wilf et al. (2000). However, based on the current fossil evidence, it is unlikely that these clades could produce the herbivory patterns in the older fossils from late Cretaceous (Kristensen and Skalski, 1999; C. C. Labandeira personal communication 2007).

The origin of Baridinae (Curculionidae), the subfamily that includes *Anopsilus* is not well established (J. Prena personal communication 2007). However the earlier fossils of Curculionidae are from the late Cretaceous (Donato et al. 2003). The basal clades of Curculionidae *sensu lato* are occupied mostly by taxa that feed on monocots, such as Zingiberales (Marvaldi et al., 2002). This suggests that curculionids (and Baridinae, if this is a basal clade of the family) are potential culprits for the oldest *Zingiberopsis* herbivory.

The ichnotaxon *C. strongii* predates the oldest known body fossil of this group by ca. 20 My (Wilf et al., 2000). If these feeding tracks were not produced by hispine beetles, this may explain some of the discrepancies between the phylogenies calibrated with this fossil and the phylogenetic analyses based on molecular data alone (Gómez-Zurita et al., 2007). Phylogenies using the fossil herbivory suggest a co-diversification of rolled-leaf beetles and its host plants during the Tertiary (McKenna and Farrell, 2006).

Phylogenetic analyses based on molecular data alone suggest a more recent origin of rolled-leaf beetles that may radiate later than its host plants (Gómez-Zurita et al., 2007).

A potential explanation for the similarity between hispine and non-hispine herbivory patterns is the presence of salt crystals and sclerified vascular bundles in the order Zingiberales (Auerbach and Strong, 1981). These features may predispose both extinct and extant Zingiberales to the convergence of stereotyped epidermal-feeding by different insect taxa (Auerbach and Strong, 1981; Jolivet and Hawkeswood 1995; García-Robledo et al. 2007). In conclusion, it is conceivable that the feeding tracks recorded in *Zingiberopsis,* as in extant Zingiberales, may belong to insect lineages other than hispines. Therefore, the fossil *Cephaloleichnites* (Hispinae) must not be solely attributed to an ancestor of the extant rolled-leaf beetles until more conclusive evidence such as the body of a fossil hispine is available.

TABLE 2.1. Extant plants from the order Zingiberales and herbivores that produce leaf damage equivalent to that described in fossils of *Zingiberopsis*. N = number of insects observed in laboratory trials.

Plant family	Plant species	N	Herbivore
Cannaceae	*Canna bangii* Kraetzl 1912	14	*Anopsilus sp. nov.* (Curculionidae, Coleoptera)
Heliconiacee	*Heliconia imbricata* Baker 1893	10	Pyralidae (Lepidoptera)
Marantaceae	*Calathea crotalifera* S. Watson 1889	8	Choreutidae (Lepidoptera)
Zingiberaceae	*Renealmia alpinia* Maas 1975 [1976]	6	Pyralidae (Lepidoptera).

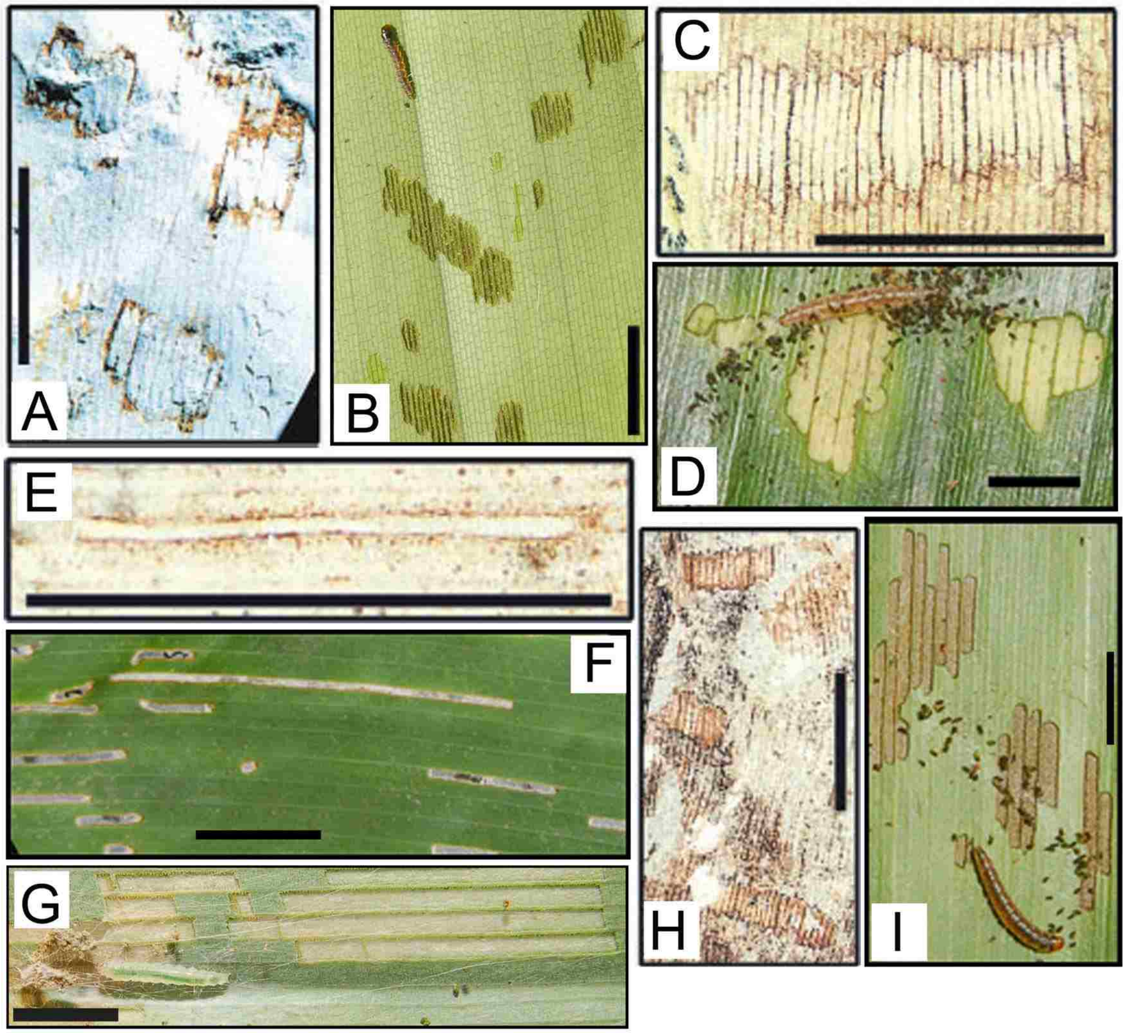

Figure 2.1. Fossil herbivory attributed to an ancestor of extant rolled-leaf beetles (*Cephaloleichnites strongi*) in *Zingiberopsis isonervosa* (Zingiberaceae) from early Eocene (A, C ,E ,H) and extant non-hispine herbivores in five families of Zingiberales (B, D ,F ,G ,I). (B). Herbivory by Pyralidae (Lepidoptera) in *Heliconia imbricata* (Heliconiaceae). (D, I) Herbivory by Pyralidae (Lepidoptera) in *Renealmia alpinia* (Zingiberales), a close relative of *Zingiberopsis*. (F) Herbivory by *Anopsilus* weevils (Curculionidae) in *Canna bangii* (Cannaceae). (G) Herbivory by Choreutidae, *Brenthia monolychna* Meyrick, 1915 (Lepidoptera) in *Calathea crotalifera* (Marantaceae). Scale bars in all panels equal 5 mm. (Photos A, C, E and H from Wilf, P., C. C. Labandeira, W. J. Kress, C. L. Staines, D. M. Windsor, A. L. Allen, And K. R. Johnson. 2000. Timing the radiations of leaf beetles: Hispines on gingers from latest Cretaceous to recent. Science, 289:291-294 with permission from AAAS). Vouchers: (A) (USNM 509718) (C) (USNM 498174) (E) (USNM 498168), (F) (USNM C. Garcia-Robledo 132–135 *Anopsilus* sp.nov.), (H) (USNM 498169)

Chapter III

Larval morphology and development, host plants, adult longevity, sexual dimorphism and notes on natural history in Cephaloleia “rolled-leaf” beetles (Coleoptera: Chrysomelidae: Cassidinae)[2]

SUMMARY

The Neotropical genus *Cephaloleia* Chevrolat, 1837 is comprised of 209 described species. Adults usually feed and mate within the scrolls formed by the young rolled leaves of plants of the order Zingiberales. This paper reports for populations of *Cephaloleia belti* Baly, *C. dilaticollis* Baly, *C. dorsalis* Baly and *C. placida* Baly at La Selva Biological Station (Costa Rica, Central America) detailed descriptions of: 1. larval and adult diets and diet breadth; 2. egg, larval and pupal morphology; 3. larval development times; 4. dimorphic sexual characteristics; 5. adult longevity; and 6. differences in lifespan between genders. *Cephaloleia belti* displays the broader diet breadth, feeding on 14 species of three families of Zingiberales. *Cephaloleia dilaticollis* feeds on nine species of three families of Zingiberales. *Cephaloleia dorsalis* and *C. placida* feed on four species of the family Costaceae and two species of the family Zingiberaceae respectively. Time to pupation ranges among species from 32.8 to 59.1 days. In the four *Cephaloleia* species, adult females are larger than males. Genders display marked sexual dimorphism in the shape of their last abdominal sternite and the pygidium. Longevity of adults ranged from 10 to 13 months. Life expectancy estimates for adult beetles reared in the laboratory ranged from 111.5 to 187.2 days. Male and female adults of *C. belti* and *C. dilaticollis* have equivalent life expectancies. However, life expectancy is longer for male *C. dorsalis.* Male *C. placida* tend to live longer than females.

BACKGROUND

The Neotropical genus *Cephaloleia* Chevrolat, 1837 is comprised of 209 described species (Staines 2008). *Cephaloleia* beetles (Tribe Cephaloleiini) are frequently referred to in the ecological literature as the “rolled-leaf” beetles. This is a guild of insect herbivores that usually feed and mate within the scrolls formed by young rolled leaves of Neotropical plants of the order Zingiberales (Strong 1981, 1982a, b, Staines 1996, 2008). Recent phylogenetic analyses revealed that the group previously known as “rolled-leaf beetles” is polyphyletic and includes the monophyletic genus *Cephaloleia* together with unrelated groups that have similar life histories, such as members of the tribe Arescini (McKenna and Farrell 2005). This paper focuses on the natural history of the monophyletic genus *Cephaloleia.* Most species of *Cephaloleia* feed on the Neotropical families Cannaceae Juss., Costaceae Nakai, Heliconiaceae Nakai, Marantaceae R. Br. and Zingiberaceae Martinov (Order Zingiberales) (Staines 1996). *Cephaloleia* additionally have been reported feeding on Bromeliaceae Juss., Cyclanthaceae Poit. ex A. Rich., Cyperaceae Juss., Orchidaceae Juss. Poaceae Barnhart (McKenna and Farrell 2005), and Arecaceae (Staines 1996, 2008).

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[2] García–Robledo, C.C. Horvitz and C.L. Staines. (in press). Adult and larval morphology, host plants, adult longevity and notes on natural history in *Cephaloleia* “rolled-leaf beetles” (Coleoptera: Chrysomelidae: Cassidinae). Zootaxa __:__-__.

The interaction between the Cephaloleiini and plants of the Zingiberales Griseb. may be one of oldest and most conservative plant-herbivore interactions (Wilf *et al.* 2000). The time of origin of this group is controversial. Estimates based on feeding tracks assumed to be produced by “rolled-leaf” beetles in Zingiberaceae from the latest Cretaceous and early Eocene suggest that ancestors of tribes Cephaloleiini or “Arescini” and Zingiberales have interacted through the last 60 million years (Wilf *et al.* 2000). However, new evidence suggests that the Cephaloleiini or Arescini are not the only potential candidates responsible for these fossil feeding tracks (García-Robledo and Staines 2008), and this beetle lineage might be at least 10-25 million years more recent than formerly thought (Gómez-Zurita *et al.* 2007).

The alpha taxonomy and phylogenetic relationships among *Cephaloleia* species have been thoroughly studied, at least for the Central American species (Staines 1996; Staines and Staines 1997; Staines 1998, 2002, 2004; McKenna and Farrell 2005, 2006; Staines 2008). During the last three decades the association between *Cephaloleia* beetles and their host plants has been a model to investigate processes underlying plant-herbivore interactions. Research on the genus *Cephaloleia* includes host plant search behavior (García-Robledo and Horvitz 2009), effects of plant chemistry on insect herbivore ecology (Auerbach and Strong 1981; Gage and Strong 1981), population dynamics (Morrison and Strong 1981; Johnson 2004a, b, 2005; Johnson and Horvitz 2005), the structure of *Cephaloleia* species assemblages (Strong 1981, 1982a, b; Descampe *et al.* 2008; Meskens *et al.* 2008), and the role of *Cephaloleia* species in micro-ecosystems such as inflorescences phytotelmata or the debris covering expanded leaves of the host plants (Seifert and Seifer 1976a, b; Seifert and Seifert 1979a, b; Seifert 1981, 1982;

McCoy 1984, 1985; Guthrie 2005). *Cephaloleia* species are also model organisms used to understand plant-herbivore evolutionary processes and the diversification of tropical insect herbivores (Strong and Wang 1977, Wilf *et al.* 2000, McKenna and Farrell 2005, McKenna and Farrell 2006, Gómez-Zurita *et al.* 2007, García-Robledo and Staines 2008).

Despite the importance of the genus *Cephaloleia* as a model to understand the ecology and evolution of tropical plant-herbivore interactions, several aspects of their development, morphology and natural history remain poorly understood. For example, host plant associations are poorly known for most species of *Cephaloleia* (Staines 2008). Detailed descriptions of larval morphology using techniques such as scanning electron microscopy are not available. In addition, details on larval development and adult life history traits are partially known for a few species.

For this research, we selected four species of the genus *Cephaloleia* with different diets and diet breadths. *Cephaloleia belti* Baly is a generalist species that feeds on plants from the families Cannaceae, Heliconiaceae and Marantaceae. *Cephaloleia dilaticollis* Baly is a generalist species that feeds on plants from the families Marantaceae and Zingiberaceae. *Cephaloleia dorsalis* Baly displays a diet restricted to plants from the genus *Costus* (Costaceae). *Cephaloleia placida* Baly displays a diet restricted to the genus *Renealmia* (Zingiberaceae) (Descampe *et al.* 2008; Meskens *et al.* 2008).

For four species of *Cephaloleia* this paper reports diet and diet breadth of larvae and adults, morphology of egg, larvae and pupae, development times of larvae under laboratory conditions, sexual characteristics and adult longevity including differences between genders. Finally, we review the literature on the ecology and evolution of

Cephaloleia beetles, and discuss how our results contribute to the current knowledge on the natural history of this plant-herbivore interaction.

METHODS

STUDY SITE — This study was conducted from August 2005 to March 2009 at La Selva Biological Station (hereafter La Selva), a tropical rain forest site in Costa Rica, Central America (10º26'N, 83º59'W). La Selva is classified as an aseasonal tropical wet forest (Holdridge 1947), receiving an average of 4000 mm rain per year (McDade *et al.* 1994). At this site there are at least 40 sympatric *Cephaloleia* species (Staines 1996) and at least 43 potential host plant species belonging to the order Zingiberales (Staines 1996; McKenna and Farrell 2005).

HOST PLANTS OF ROLLED-LEAF BEETLES — We recorded the host plant species of larvae and adults of *C. belti* (Fig. 3.1A)*, C. dilaticollis* (Fig. 3.1H)*, C. dorsalis* (Fig. 3.1L) and *C. placida* (Fig. 3.1P) at La Selva. We censused 2066 individual plants belonging to 32 species of Zingiberales and recorded the presence and identity of *Cephaloleia* larvae and the plant structure where larvae were observed. We also recorded the number of adults of the four beetle species within the scroll formed by the young-rolled leaves. Each rolled leaf was collected from a different plant. The minimum distance between plants was 2 m. To describe the range of the diet and the frequency of host use by adults, we calculated the mean number of individuals of *C. belti, C. dilaticollis, C. dorsalis* and *C. placida* in each host plant.

LARVAL MORPHOLOGY — Larvae were collected into and maintained in 95% ethanol (EtOH). Some specimens used for SEM imaging were further dehydrated using 95% and 100% EtOH, three times for five minutes each and a final dehydration using

HMDS (Hexamethyldisilazane) for 5 minutes, three times. Specimens were mounted on carbon adhesive tabs on aluminum stubs. Samples were sputter coated using Palladium for 3 minutes. SEM imaging was achieved using FEI XL-30 ESEM-FEG. Vouchers of larval stages and the mounted specimens used for SEM imaging were deposited in the Department of Entomology collection, US National Museum of Natural History (NMNH).

EGG LARVAL AND PUPAL DEVELOPMENT — Females of *C. belti, C. dilaticollis, C. dorsalis* and *C. placida* were collected in the wild and brought to the laboratory to obtain eggs for morphological descriptions. Females were placed with a male and leaf tissue from their host plant in individual containers. Where beetle species were known to have more than one host plant, we selected the plant species where both larvae and adults had been recorded, and adult beetles were most frequently observed (Figs.17 to 20). For each egg, we measured its length and width and time to larval eclosion (*see* sample sizes in Table 1).

To describe larval and pupal development in the four focal species, we placed newly eclosed larvae in individual containers (*see* sample sizes in Table 3.1). Larvae were reared at a mean temperature of 27°C and a light regime of 12 h. light 12 h. darkness. Larvae were fed each 24 h. with two 3.5 cm diameter disks of leaf tissue from expanded leaves of their host plants. In cases where beetle species had more than one host plant, we selected the plant species where larvae had been recorded and adult beetles were most abundant in the field (Figs. 3.2A to 3.2D).

We measured the length of larvae using a digital camera (Diagnostic Instruments Inc. Model 3.2.0) attached to a stereoscope (Leica MZ 12s). Lengths of the larvae were estimated on the digital images at an accuracy of 1 X 10^{-2} mm, using the program Spot V.3.5.8 (Diagnostic Instruments Inc. Sterling Heights, MI).

For each beetle species we also recorded the date of each molt and the time to pupation. Pupa length and weight were measured on the day of pupation. Weight was measured using an analytic balance Scientech SA 40 with a precision of 10^{-4} g. We also recorded the time from pupation to eclosion.

SEXUAL DIMORPHISM —The length of each adult from the tip of the head to the end of the last abdominal segment was measured as adults emerged from laboratory-reared pupae. In addition, we measured adult weight. Differences in length and weight between genders were tested for each beetle species using Mann-Whitney *U* tests. In addition, we describe a useful characteristic to recognize each gender, sexual dimorphism in the morphology of the last abdominal segment.

ADULT LONGEVITY —To determine the longevity of adults, and to explore differences in lifespan between genders, we placed unmated adult beetles reared in the laboratory in independent containers (*see* sample sizes in Table 3.1). Adults were fed *ad libitum* every 48 h with leaf tissue from fresh young rolled leaves, recording the total days alive. (Host plants: *C. belti* on *Heliconia latispatha* (Heliconiaceae), *C. dilaticollis* on *Renealmia alpinia* (Zingiberaceae), *C. dorsalis* on *Costus malortieanus* (Costaceae), *C. placida* on *Renealmia alpinia* (Zingiberaceae)). Differences in longevity between genders were tested for each species using Kaplan-Meier survival analyses (Crawley 2007).

RESULTS

HOST PLANTS OF ROLLED-LEAF BEETLES — Larvae of *C. belti* were observed in *Heliconia latispatha*, *H. imbricata* (Kuntze) Baker and *H. wagneriana* Petersen. Larvae feed on the inner area of the petioles, the base of young rolled leaves, and on expanded leaves. Larvae feeding on expanded leaves are usually found under the humid shelter of the leaf litter covering leaves of its host plants. Larvae are sometimes found in *Heliconia* young-rolled leaves, especially in *H. wagneriana*.

Larvae of *C. dilaticollis* were observed in *Renealmia alpinia* (Rottb.) Maas. Larvae feed on the petioles and blades of fully expanded leaves.

Larvae of *C. dorsalis* were observed in *Costus malortieeanus* and *C. laevis* Ruiz & Pav. Larvae of *C. dorsalis* feed on young and fully expanded leaves, and on the bracts of inflorescences. First instar larvae feeding on pubescent host plants such as *C. malortieeanus,* may cut plant hairs and cover their backs with them.

Cephaloleia placida larvae were observed in *Renealmia alpinia*. *Cephaloleia placida* larvae feed on leaf tissue from the petioles and the leaf sheaths of expanded leaves. Larvae also feed on the bracts subtending the inflorescences and infructescences of *R. alpinia.*

The adults of the four species of *Cephaloleia* beetles included in this study differ in their diet breadth and diet composition. Adult *C. belti* display a broad diet, feeding on at least 15 host plants. Individuals of *Cephaloleia belti* were recorded on plants from the families Cannaceae, Heliconiaceae and Marantaceae (Fig. 3.2A). Adults of *C. dilaticollis* were recorded in at least nine plant species from the families Heliconiaceae, Marantaceae and Zingiberaceae (Fig. 3.2B). Adult *C. dorsalis* were recorded in four species of the genus *Costus* (Costaceae). One individual of *C. dorsalis* was recorded on *Calathea leucostachys*

Hook (Marantaceae) (Fig. 3.2C). Adults of *C. placida* were recorded on rolled leaves of the neotropical gingers *Renealmia alpinia* and *Renealmia cernua* (Sw. ex Roem. & Schult.) J.F. Macbr. (Fig. 3.2D).

LARVAL MORPHOLOGY OF *CEPHALOLEIA* CHEVROLAT BEETLES — In general, larvae of *Cephaloleia* Chevrolat are rounded, oval, longer-than-wide, with even, regular margins formed by wide expansion of all segments from prothorax to caudal abdominal segment forming a scale-like shield; head and legs concealed by broadly flattened margins; expansions extending far forward in front of the head for a distance much greater than the width or length of the head, beyond the thorax at the sides to a width greater than ½ the width of the body proper and beyond the abdomen at the sides to a width wider on each side than the width of abdomen proper, width at caudal end nearly as great as at anterior end; expansions narrowly laminate; segments more or less distinct, sides plicate; elevated along central longitudinal medial line which is wider after the middle to the prothorax and narrows on tergites 7-9. Divisions between the head and the prothorax and abdominal tergites 7-9 are not clearly defined. Dorsal surface convex. Head retracted; antenna with three antennomeres. Legs consist of two distinct segments plus base; ending with a single, strong, recurved claw. In the following larvae descriptions, measurements were taken with an ocular micrometer. Total length was measured from the anterior to the posterior margins. Total width was measured at the widest point.

***Cephaloleia belti* Baly -** Color when live (Fig. 3.1B) is yellowish-brown with outer margins translucent; center portion reddish with some yellowish areas especially near head. Color when dead is pale-brown centrally, margins paler becoming almost transparent at edge; venter paler than dorsum. Dorsum carries a longitudinal medial setose ridge extending from anterior to posterior margin (Fig. 3.3A). Total length 6.7 mm (n=1); width 4.3 mm.

Dorsum. Prothorax with central area raised, micropunctate, with dark setae on either side of medial longitudinal ridge and on basal slope; lateral areas rugose; two diagonal carinae on central raised area extending to anterior margin. Mesothorax with central raised portion with shallow v-shaped carina; laterally with sharply curved carina which extends to lateral margin. Metathorax with central portion irregularly plicate; with transverse carina extending across entire width. Abdominal tergites 1-6 slightly narrowed in middle, wider at sides; with transverse carina in middle of each side; spiracle near basal margin on each side just off central elevation; spiracles appear as spot with dark margin, orifice as in Fig. 3.3B. Abdominal tergites 7-10 with surface plicate; with three carinae along margin on each side.

Venter. Surface of expansions punctate, rugose-striate. Head (Fig. 3.3C) surface rugose-punctate; labrum with surface alutaceous, without setae; clypeus with fringe of long setae at apex, with four setae on apical ½, surface alutaceous; mandibles tridentate; maxillary palps with 2 palpomeres and 12 short, robust setae at apex; maxilla robust, clavate, with fringe of long setae at apex; labium densely setose. Antenna with antennomere 1 short, robust; 2 wider than 1, transverse; 3 elongate, cylindrical, subequal in length to 1 and 2 combined, with fringe of short setae at apex. Pro- and mesothorax wider than long; slightly depressed in middle; surface rugose-striate. Metathorax longer than others; depressed in middle; with suture along apical margin. Abdominal sternites

1-7 wider than long; decreasing in width; with three sulci on apical ½; laterally with curved sulcus dividing the sternite into thirds; sternite 8 similar to preceding but without any sulci; sterna 9-10 fused, rounded at apex. Leg: segment 1 wider and shorter than 2; segment 2 subconical, with a strong claw and eight setae at apex (Fig. 3.3D).

***Cephaloleia dilaticollis* Baly -** Color when live (Fig. 3.1F) pale yellowish-brown, margins translucent; which dark markings as follows: posterior margin and small medial spot on prothorax; mesothorax with medial longitudinal stripe and basal margin; metathorax and abdominal tergite 1 with medial longitudinal stripe and anterior and posterior margins; abdominal tergites 2-4 with medial longitudinal stripe; tergites 6-7 similar to metathorax; tergites 8-10 with medial longitudinal stripe. Venter pale yellowish. Color when dead pale yellowish with dark markings. With medial longitudinal ridge from anterior to posterior margin. Total length 5.7-6.0 mm (n=2); width 3.6 mm.

Dorsum. Prothorax with central raised area, surface micropustulate; with two diagonal carinae from central raised area to anterior margin; anterior and lateral areas punctate. Mesothorax with anterior margin carinate from side to side; laterally with sharply curved carina which extends to anterior margin; punctate laterally. Metathorax with diagonal carina which extends to lateral margin; punctate laterally. Abdominal tergites 1-6 wider than long, decreasing in width; punctate laterally. Tergites 7-9 with two diagonal carinae on each side which extend to lateral margins. Spiracles just off central elevation, with margins darkened; orifice as in Fig. 3.4A.

Venter. Surface of expansions rugose-punctate. Head (Fig. 3.4B) surface punctate; labrum with surface alutaceous, without setae; clypeus with fringe of long setae at apex, with four setae on apical ½, surface alutaceous; mandibles tridentate; maxillary palps with 2 palpomeres and 12 short, robust setae at apex; maxilla robust, clavate, with fringe of long setae at apex; labium densely setose. Antenna with antennomere 1 longer than 2, subcylindrical; 2 wider than 1, transverse; 3 obconical, slightly longer than 2, with short setae at apex. Prothorax nearly as wide as long. Meso- and metathorax wider than long. Abdominal sternites 1-7 wider than long; decreasing in width; with three sulci on apical ½; laterally with curved sulcus dividing the sternite into thirds; sternite 8 similar to preceding but without any sulci; sterna 9-10 fused, rounded at apex. Leg (Fig. 3.4C) robust; segment 1 rugose-striate, with scattered setae; 2 obconical, with scattered long setae and strong claw at apex.

Cephaloleia dorsalis **Baly-** Color when live (Fig. 3.1J) pale yellowish, margins translucent; venter pale yellowish. Color when dead dirty-brown with paler margins. Total length 6.4-6.7 mm (n=3); width 4.3-4.4 mm.

Dorsum. Prothorax surface of central elevation micropustulate; with two diagonal carinae laterally extending to lateral margin, carinae wide at base, narrowing apically to sharp point; surface laterally punctate. Meso- and metathorax with base elevated, sloping back to apex, narrowing laterally into sharp point; punctate laterally. Abdominal tergites 1-6 wider than long, decreasing in width; base elevated, sloping back to apex, ending in sharp point; punctate laterally. Tergites 7-9 with two diagonal carinae on each side which extend to lateral margin. Spiracles just off central elevation, with margins darkened; orifice as in Fig. 3.5A.

Venter. Surface of expansions punctate, rugose-striate. Head (Fig. 3.5B) surface rugose-punctate; labrum with surface alutaceous, without setae; clypeus with fringe of long setae at apex, with four setae on apical ½, surface alutaceous; mandibles tridentate; maxillary palps with 2 palpomeres and short, robust setae at apex; maxilla robust, clavate, with fringe of long setae at apex; labium densely setose. Antenna with antennomere 1 short, robust; 2 narrower than 1, transverse; 3 elongate, cylindrical, narrower than 2, with fringe of short setae at apex. Pro- and mesothorax wider than long; slightly depressed in middle; surface rugose-striate. Metathorax longer than others; depressed in middle; with suture along apical margin. Abdominal sternites 1-8 wider than long; decreasing in width; laterally with curved sulcus dividing the sternite into thirds; sterna 9-10 fused, rounded at apex. Leg: segment 1 short, robust; segment 2 subconical, with a strong claw and eight setae at apex (Fig. 3.5C).

Cephaloleia placida **Baly -** Color when live (Fig. 3.1N) brownish-yellow with body proper reddish, margins translucent; venter paler. Color when dead dirty-brown with paler margins. Dorsum with longitudinal medial setose ridge extending from anterior to posterior margins (Fig. 3.1N). Total length 7.3 mm (n=1); width 4.4 mm.

Dorsum. Prothorax without diagonal carinae on central raised area; central area slightly raised, micropunctate; lateral areas micropunctate. Mesothorax without carinae, micropunctate. Metathorax with transverse carina in middle of each side. Abdominal tergites 1-6 slightly narrowed in middle; with transverse carina in middle of each side just off central elevation; spiracles appear as darker brownish spot without darker margin, orifice as in Fig. 3.6A. Abdominal tergites 7-10 with two carinae along margin on each side; surface micropunctate.

Venter. Surface of expansions rugose-punctate. Head (Fig. 3.6B) surface punctate; clypeus slightly rugose, with fringe of setae at apex; mandibles tridentate; maxillary palps with 2 palpomeres and short, robust setae at apex; maxilla robust, clavate, with fringe of long setae at apex; labium densely setose. Antenna with antennomere 1 short, robust; 2 elongate, cylindrical, longer than 3; 3 cylindrical, with fringe of short setae at apex. Pro- and mesothorax wider than long; slightly depressed in middle; surface rugose-striate. Metathorax longer than others; depressed in middle; with suture along apical margin. Abdominal sternites 1-8 wider than long; decreasing in width; laterally with curved sulcus dividing the sternite into thirds; sterna 9-10 fused, rounded at apex. Leg: segment 1 short, robust; segment 2 subconical, with a strong claw and eight setae at apex (Fig. 3.6C).

EGG LARVAL AND PUPAL DEVELOPMENT — *Cephaloleia belti* and *C. dilaticollis* lay eggs on the petioles and rolled leaves of their host plants. Both species lay eggs singly or in clusters of 2 or more (Figs. 3.1A,E). During oviposition, eggs can be covered with frass. *Cephaloleia dorsalis* usually lays eggs singly on the leaf petioles, the surface of bracts covering the stalk of its host plants, or in the inner surface of inflorescence bracts (Fig. 3.1I). *Cephaloleia placida* lays eggs singly or in clusters of 2 or more in the concavity of leaf petioles or the inner surface of inflorescence bracts (Fig. 3.1M).

Cephaloleia belti and *Cephaloleia dilaticollis* eggs are smaller than eggs of *C. dorsalis* and *C. placida.* Time to eclosion ranges from 7.0 to 12.5 days (Table 3.1).

Newborn larvae are white and translucent. Larval size after emergence varied from ca. 2.0 - 2.1 mm in *C. belti* and *C. dilaticollis* to ca. 2.3-2.6 mm in *C. dorsalis* and *C. placida* (Table 3.1). All species molted only once. Molting time ranged among species between 11.4 to 25.9 days (Table 3.1).

After molting, larval color changed in the four beetle species. Second instar larvae of *C. belti* turned pale orange (Fig. 3.1B). The second instar of *C. dilaticollis* larvae displayed a distinctive dorsal black pattern (Fig. 3.1F). Second instar larvae of *C. dorsalis* were pale red after molting, and turned dark red in the center of the dorsal area when approaching pupation (Fig. 3.1J). The second instar of *C. placida* was pale orange (Fig. 3.1N).

Larvae of the four species pupated inside the second instar larval exhuvia. Pupae of each beetle species display distinctive ornaments (Fig 3.1C, G, K and O).
Time to pupation varies among species, ranging between 1 to 2 months (Table 3.1). Pupal length and weight ranges from 5.6-7.4 mm and 5.7×10^{-3} – 1.46×10^{-2} g, respectively (Table 3.1).

SEXUAL DIMORPHISM — In the four beetle species, females are larger and heavier than males (Table 3.1). (*Cephaloleia belti*: length, $U = 827.5$, $N_{females} = 101$, $N_{males} = 109$, $P < 0.001$, weight $U = 794$, $N_{females} = 101$, $N_{males} = 109$, $P < 0.001$; *C. dilaticollis*: length, $U = 94$, $N_{females} = 38$, $N_{males} = 36$, $P < 0.001$, weight $U = 182$, $N_{females} = 38$, $N_{males} = 36$, $P < 0.001$; *C. dorsalis*: length, $U = 227$, $N_{females} = 41$, $N_{males} = 45$, $P < 0.001$, weight $U = 242$, $N_{females} = 40$, $N_{males} = 45$, $P < 0.001$; *C. placida*: length, $U = 69.5$, $N_{females} = 32$, $N_{males} = 38$, $P < 0.001$, weight $U = 59$, $N_{females} = 32$, $N_{males} = 38$, $P < 0.001$).

The four beetle species display marked sexual dimorphism in the shape of the last abdominal sternite and the pygidium. The end of the last sternite in males of all four species is u-shaped, least so in *C. belti*, while in *C. placida* it has a u-shaped concavity covered by an ovoid pygidium (Figs 3.7A-D). In females of *C. belti,* the last sternite is slightly acuminate (Fig. 3.7A), in *C. dilaticollis* is truncate (Fig. 3.7B), in *C. dorsalis* is ovoid (Fig. 3.7C) and in *C. placida* is slightly acuminate (Fig. 3.7D).

ADULT LONGEVITY — The longevities of unmated male and female *C. belti* are equivalent (df = 1, χ^2 = 0.4. P = 0.54). Average life expectancy for *C. belti* is 117 days, but adults did live for up to 297 d (Days alive females: Mean ± SD = 120.5 ± 46.9, Min - Max = 7 -220; Days alive males: Mean ± SD = 113.3 ± 86.1 Min - Max = 5 – 297, Fig. 3.8A).

Lifespans of unmated male and female *C. dilaticollis* are equivalent. (df = 1, χ^2 = 0.3. P = 0.61). Average life expectancy for *C. dilaticollis* was 168.9 d, however adults did live for up to 297 d (Days alive females: Mean ± SD = 174.2 ± 70.6 , Min - Max = 22 - 297; Days alive males: Mean ± SD = 164.1 ± 67.7, Min - Max = 2 – 254, Fig. 3.8B).

Genders of unmated *C. dorsalis* display significant differences in lifespan. Males live 25% longer than females. (df = 1, χ^2 = 4.3. P = 0.038). Adult *C.dorsalis* lived for up to 338 d (Days alive females: Mean ± SD = 157.2 ± 90.1, Min - Max = 6 - 294 ; Days alive males: Mean ± SD = 210.4 ± 83.7, Min - Max = 6 – 338 , Fig. 3.8C). Life expectancy of *C. dorsalis* is 187.2 d.

Males of unmated *C. placida* lived 19% longer than females (df = 1, χ^2 = 3.8. P = 0.05). Adult *C. placida* can live up to 391 d. (Days alive females: Mean ± SD = 102.3 ± 66.0, Min - Max = 6 - 253 ; Days alive males: Mean ± SD = 127.0 ± 113.9 , Min - Max = 4 – 391, Fig. 3.8D). Life expectancy of *C. placida* is 111.5 d.

DISCUSSION

HOST PLANTS OF ROLLED-LEAF BEETLES — It is frequently assumed that "rolled-leaf beetle" larvae feed only on young rolled leaves (Wilf *et al.* 2000). This is true for some *Cephaloleia* species. For example, at La Selva, larvae of *Cephaloleia erichsonii* Baly had been recorded only in young rolled leaves of *Calathea gymnocarpa* H. Kenn. and *Calathea inocephala* (Kuntze) H. Kenn. & Nicolson (Marantaceae) (C. García-Robledo, unpubl. dat.). However, current records suggest that larval diets greatly vary among species of *Cephaloleia*.

Some *Cephaloleia* larval diets may be restricted to particular host plant structures. For example, larvae of *Cephaloleia fenestrata* Weise feed on tissue from the petiolar concavities of its only known host, *Pleiostachya pruinosa* (Regel) K. Schum. (Marantaceae) (Johnson 2004a). *Cephaloleia* larvae can also specialize on bracts of *Heliconia* inflorescences. Larvae of *C. puncticollis* Baly feed on floral bracts of *Heliconia imbricata* (Heliconiaceae) (Guthrie 2005). Larvae of *C. neglecta* Weise feed on inflorescence bracts of *H. bihai* (L.) L. and *H. aurea* G. Rodr. (Seifert and Seifert 1979b).

Some larvae of *Cephaloleia* feed on totally expanded leaves. At La Selva, for instance, *C. belti* and *C. dorsata* Baly larvae generally feed on expanded *Heliconia imbricata* leaves, beneath a leaf, which has fallen from an overhead plant (Auerbach and Strong 1981; McCoy 1984, 1985). We recorded larvae of *C. dilaticollis* also feeding on expanded leaves of the Neotropical ginger *Renealmia alpinia*. Larval *C. belti* and *C.*

dorsalis feed on both totally expanded and young leaves of their host plants. Larvae of some *Cephaloleia* feed on both leaves and reproductive structures of host plants. We found that *C. dorsalis* and *C. placida*, in addition to feeding on leaves, also feed on inflorescence bracts of their host plants.

The four beetle species included in this study use as hosts Neotropical Zingiberales. Most of the plants reported as hosts of *Cephaloleia* beetles feed on plants from the order Zingiberales (Staines 1996; Descampe *et al.* 2008; Meskens *et al.* 2008). However, there are some records of *Cephaloleia* beetle species feeding on plants from the families Arecaceae, Bromeliaceae, Cyclanthaceae, Cyperaceae, Orchidaceae and Poaceae (McKenna andFarrell 2005; Garcia-Robledo unpubl. data, Sandino 1972). In a phylogeny of the genus *Cephaloleia,* species feeding on the plant families Arecaceae and Cyclanthaceae are grouped in a basal clade, the 'Arecaceae-feeding Clade' *sensu* McKenna and Farrell 2005. The 'Arecaceae-feeding Clade' also includes species from the Cassidinae genera *Demotispa*, *Imatidium* and *Pseudostilpnaspis* (McKenna and Farrell 2005). Species included in this basal clade display a combination of unusual morphological features (McKenna and Farrell 2005). Several species within the 'Arecaceae-feeding Clade' were recently transferred from *Cephaloleia* to different genera of Cassidinae (Staines 2009b).

Records of species of *Cephaloleia* including plants from different orders in their diets are rare (McKenna and Farrell 2005, Meskens *et al.* 2008). At La Selva, we have only recorded only one beetle species feeding on more than one plant order, *C. trivittata* Baly, which feeds on several species of Marantaceae (order Zingiberales) and the grass *Pharus latifolius* L. (family Poaceae, order Poales) (C. García-Robledo, unpubl. data).

LARVAL MORPHOLOGY — Larval morphology was discussed in Jolivet (2003) for *C. puncticollis* Baly and *C. neglecta* Weise where adults feed in the rolled leaves of host plants while the larvae are only found in the semi-aquatic inflorescences of *Heliconia*. Larvae of *Cephaloleia* in some cases may display a setose venter apparently associated with semi-aquatic life habitat (Jolivet 2003). In the four species of *Cephaloleia* included in this study, larvae do not have a setose venter.

We also note that the larva described as *C. belti* by Maulik (1932) does not resemble the larvae of *C. belti,* or any other *Cephaloleia* larva that we have examined. The description and illustrations more closely resemble larvae of the genus *Chelobasis* Gray (Tribe Arescini) (Staines 2009a). Examination of Maulik's material is necessary to resolve this apparent conflict.

EGGS, LARVAL AND PUPAL DEVELOPMENT — The times to eclosion recorded by this study are similar to those reported for *Cephaloleia fenestrata* (Johnson 2004a). The species of *Cephaloleia* included in this study molted only once. Similar results were reported for other *Cephaloleia* species (Auerbach and Strong 1981; Johnson 2004a). Larval development times in this study ranged from 49 days in *C. dilaticollis* up to 80 days in *C. placida.* Larval development is rapid in some *Cephaloleia* species, such as *C. neglecta,* with a total development time of 32 days (Seifert and Seifert 1979a, b), but long in other species. For example, *Cephaloleia fenestrata* larval development extends to ca. 125 days (Johnson 2004a). In general, our results agree with other studies suggesting that larval development in beetles from the genus *Cephaloleia* is longer than in other chrysomelid species (Strong and Wang 1977).

SEXUAL DIMORPHISM — Females are longer and heavier than males in the four *Cephaloeia* species included in this study. For insects that display size sexual dimorphism, females are usually larger than males (Teder and Tammaru). Similar differences in size between males and females was observed in for *Cephaloleia fenestrata* (Johnson 2004a). Sexual dimorphism is also present in the last abdominal sternite of each of the study species as described for other *Cephaloleia* species (Uhmann 1942). We did not recognize other obvious external features associated with sexual dimorphism in *Cephaloleia* beetles commonly found among chrysomelid species (*e.g.* Waloff and Richards 1957; Adams and Funk 1997; Emlen 2008).

ADULT LONGEVITY — The lifespan for adult beetles recorded in this research ranged from 10 to 13 months. The four species of *Cephaloleia* don't display diapause and are iteroparous (*i.e.* they mate and reproduce throughout their lives). The maximum lifespan of the adults is similar to other iteroparous chrysomelid species (Bartlett and Murray 1986). Chrysomelid species that diapause may display longer lifespans than beetles from the genus *Cephaloleia*. For example, the lifespan of Colorado potato beetles (*Leptinotarsa decemlineata* (Say)) displayed diapause may be extended up to 770 days (Bartlett and Murray 1986).

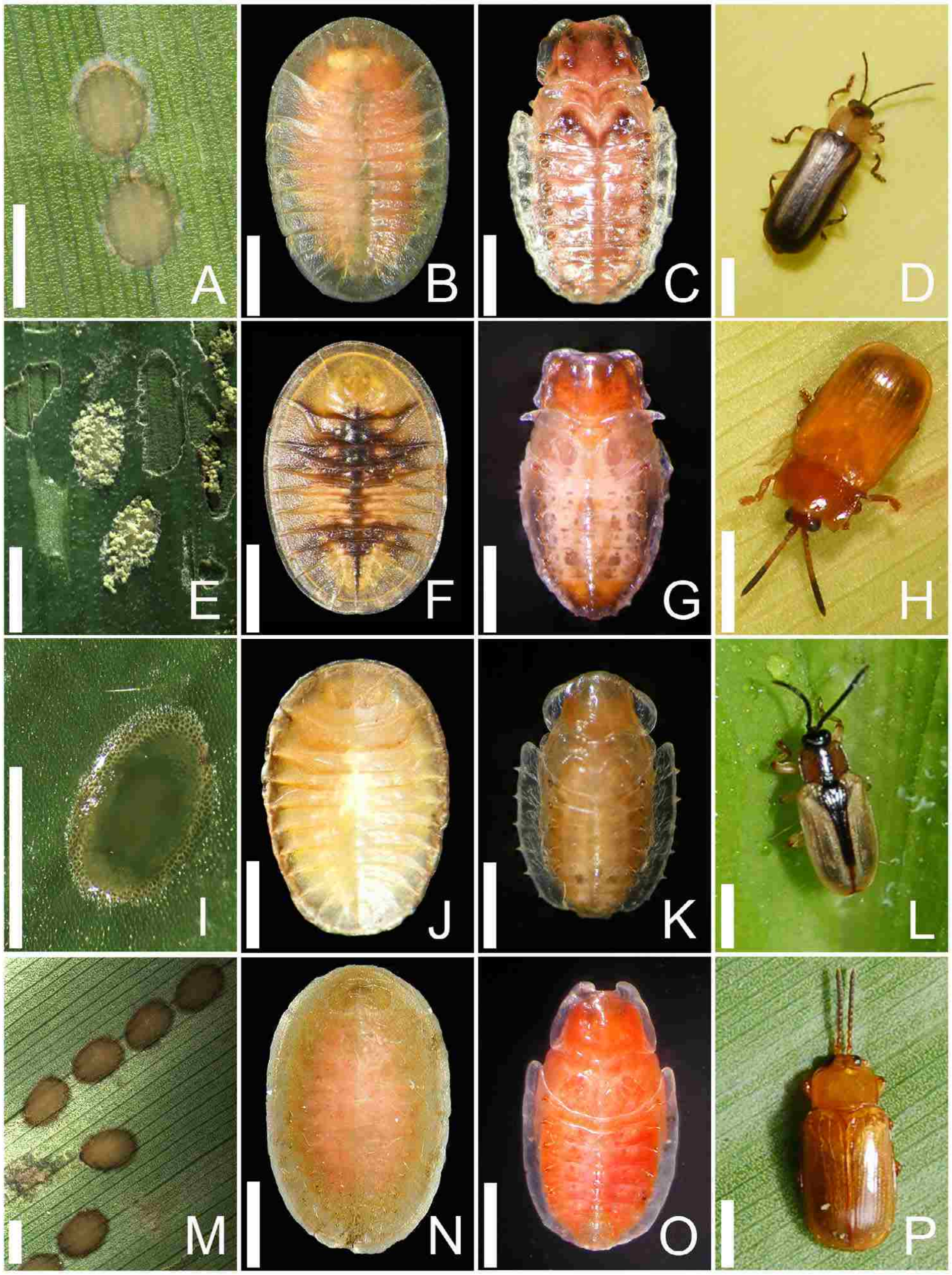
In this study, life expectancy estimates for adult beetles reared in the laboratory ranged from 111.5 to 187.2 d. Outside the current study, life expectancy has been estimated for Cephaloleiini only for *Cephaloleia fenestrata* (Johnson 2004a) where average adult life expectancy is only 68 days (Johnson 2004a). However, life expectancy for *C. fenestrata* was estimated in the field, using mark-recapture models. Estimates of life expectancy in the field integrate factors increasing mortality obviously not included in our laboratory study, such as predation, food scarcity and diseases. In addition, adults included in our experiments also did not mate. This reduction of reproduction could increase adult longevity in the laboratory. Although it is possible that the intrinsic life expectancy is shorter for *C. fenestrata* than for the four beetle species included in this study, differences between estimates are more likely a consequence of the exclusion of several mortality factors from our laboratory trials.

Our results suggest that life expectancy varies between genders for some species of *Cephaloleia* and not in others. In this study males and females of *C. belti* and *C. dilaticollis* had equivalent life expectancies. However, life expectancy is longer for males in *C. dorsalis* and *C. placida*. Estimates of life expectancy in the field for *Cephaloleia fenestrata* also show that males display longer life expectancy than females (Johnson 2004a, b). In most species females have a longer life expectancy than males (Smith and Warner 1989)**.** However, as shown by this and other studies, there are several exceptions where males live longer than females (Fox *et al.* 2003).

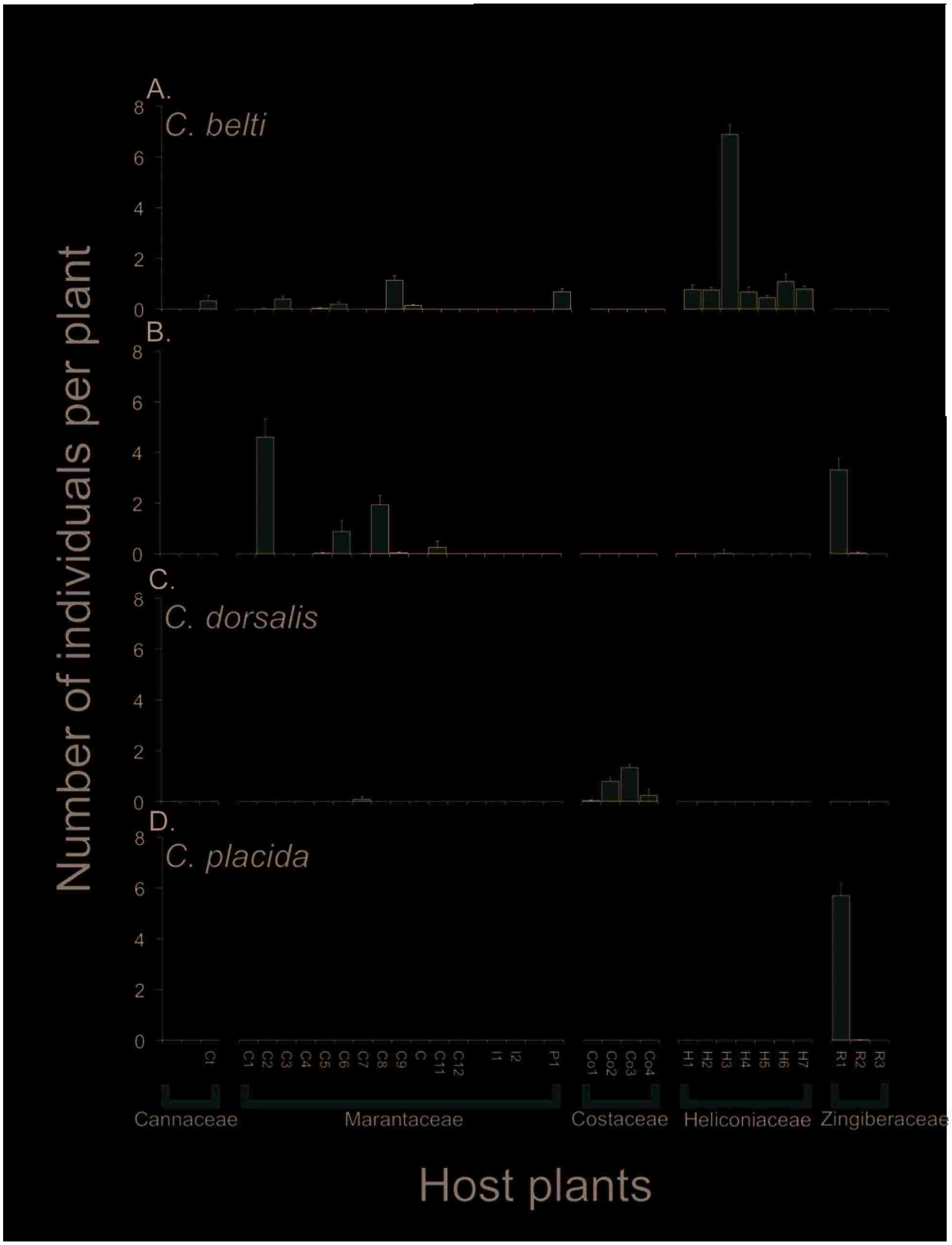
In conclusion, this study illustrates the high variation in larval and adult diet breadth among *Cephaloleia* species. Our results also show considerable diversity in morphology, development and demography among sympatric *Cephaloleia* species.

Table 3.1.Egg, larvae and pupae sizes, development times and size sexual dimorphism in four species of rolled-leaf beetles reared in their native host plants. Host plants selected to rear *Cephaloleia* species: *C. belti* in *Heliconia latispatha* (Heliconiaceae), *C. dilaticollis* in *Renealmia alpinia* (Zingiberaceae), *C. dorsalis* in *Costus malortieanus* (Costaceae), *C. placida* in *Renealmia alpinia* (Zingiberaceae).

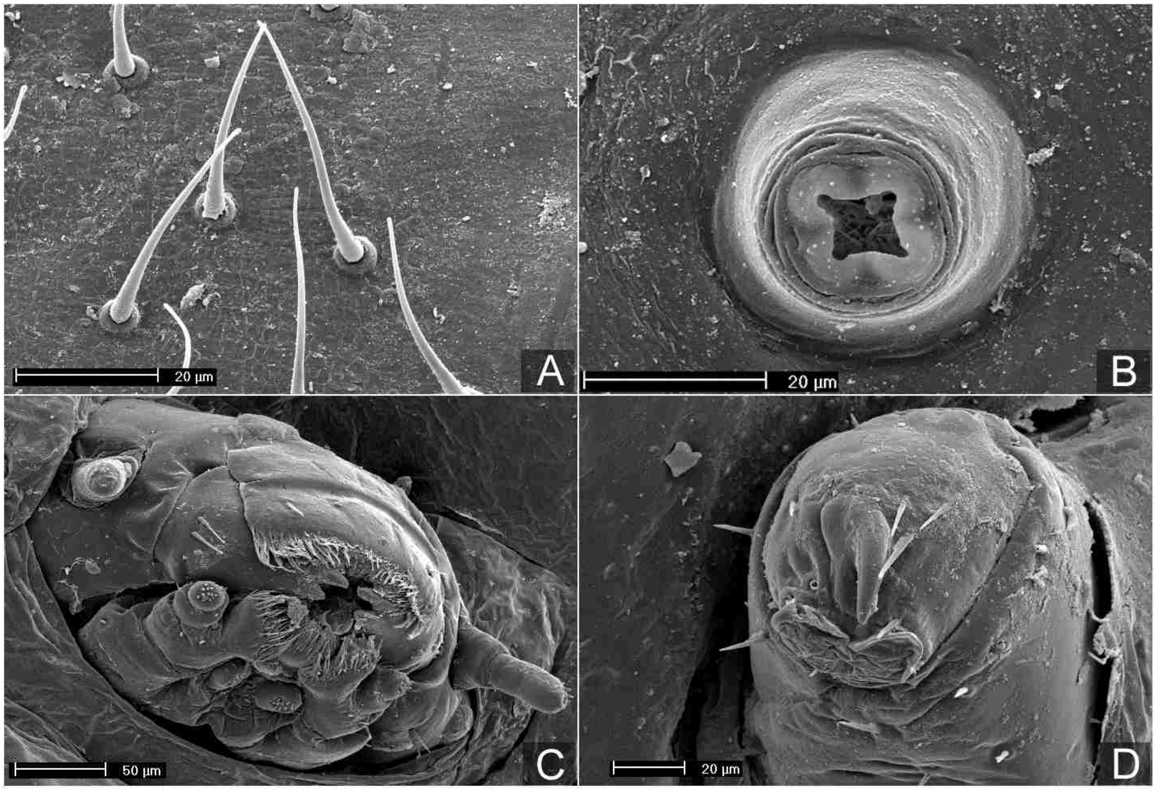
	C. belti			*C. dilaticollis*			*C. dorsalis*			*C. placida*		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Eggs												
Length (mm)	1.95	0.13	21	2.20	0.18	50	2.43	0.12	54	2.66	0.18	50
Width (mm)	1.47	0.11	21	1.20	0.14	50	1.65	0.13	54	1.73	0.16	50
Time to eclosion (d)	6.96	0.44	27	7.37	2.04	50	12.48	2.12	52	11.10	2.06	50
Larvae												
Length newborn larvae (mm)	2.149	0.103	1143	2.029	0.136	1337	2.262	0.112	663	2.611	0.113	732
Time to first molt	12.00	1.88	175	11.42	3.04	169	14.53	4.00	66	25.90	9.38	78
Pupae												
Development time larva to pupa (d)	39.151	7.177	278	32.804	8.246	143	42.791	6.688	115	59.121	16.584	91
Pupae length (mm)	7.372	0.321	278	5.799	0.336	142	6.445	0.388	116	7.063	0.407	91
Pupae weight (g)	0.0132	0.0019	278	0.0057	0.0009	142	0.0097	0.0017	116	0.0146	0.0027	91
Adults												
Development time pupa-adult (d)	16.276	1.012	210	16.568	3.079	74	17.690	3.958	84	17.817	2.219	71
Female length (mm)	6.486	0.258	101	4.294	0.164	38	5.486	0.343	41	6.240	0.335	32
Male length (mm)	5.986	0.214	109	3.927	0.172	36	5.015	0.235	45	5.475	0.224	38
Female weight (mm)	0.0089	0.0008	101	0.0044	0.0005	38	0.0069	0.0011	40	0.0109	0.0015	32
Male weight (mm)	0.0073	0.0007	109	0.0036	0.0005	36	0.0054	0.0006	45	0.0079	0.0009	38



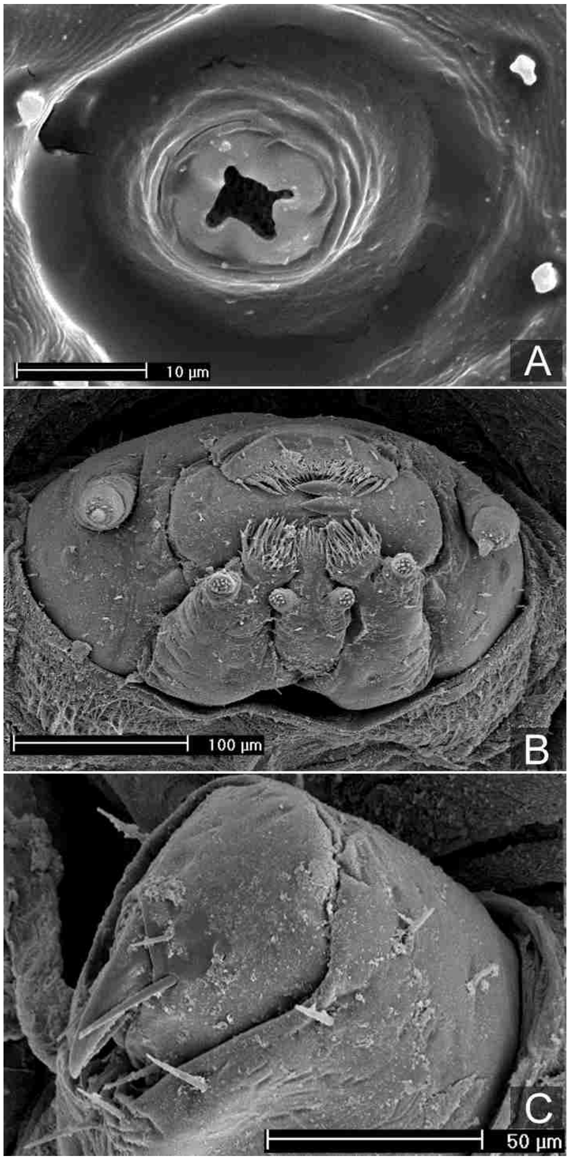
Figures 3.1. Eggs, larva, pupae and adults of four species of rolled-leaf beetles. A-D. *Cephaloleia belti*. E-H. *C. dilaticollis.* I-L. *C. dorsalis*. M-P. *C. placida.* Scale bars in all panels equal 2 mm.


A.
C. belti
B.
C.
C. dorsalis
D.
C. placida
8
6
4
2
0
Number of individuals per plant
Ct
C1
C2
C3
C4
C5
C6
C7
C8
C9
C
C11
C12
I1
I2
P1
Co1
Co2
Co3
Co4
H1
H2
H3
H4
H5
H6
H7
R1
R2
R3
Cannaceae
Marantaceae
Costaceae
Heliconiaceae
Zingiberaceae
Host plants

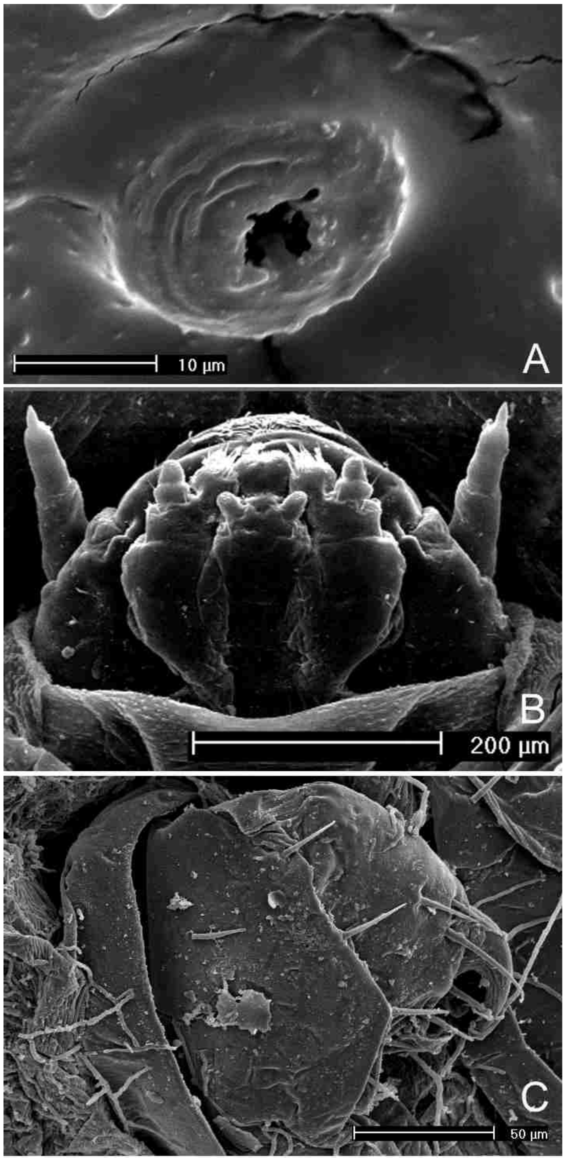
Figures 3.2. Diet breadth and mean ± SD number of adult beetles per plant. A. *Cephaloleia belti.* B. *C. dilaticollis*. C. *C. dorsalis*. D. *C. placida.* Ct: *Canna tuerckheimii* Kraenzl, C1: *Calathea cleistantha* Standl., C2: *C. crotalifera* S. Watson, C3: *C. gymnocarpa* H. Kenn., C4: *C. hammeli* H. Kenn., C5: *C. inocephala inocephala* (Kuntze) H. Kenn. and Nicolson, C6: *C. lasiostachia* Donn. Sm., C7: *C. leucostachys* Hook. f., C8: *C. lutea* Schult., C9: *C. marantifolia* Standl., C10: *C. micans* (L. Mathieu) Körn., C11: *C. similis* H. Kenn., C12: *C. warscewiczii* (L. Mathieu ex Planch.) Planch. & Linden, I1: *Ischnosiphon elegans* Standl., I2: *I. inflatus* Standl., P1: *Pleiostachya pruinosa* (Regel) K. Schum., Co1: *Costus bracteatus* Rowlee, Co2: *C. laevis* Ruiz & Pav., Co3: *C. malortieanus* H. Wendl., Co4: *C. pulverulentus* C. Presl, H1: *Heliconia imbricata* (Kuntze) Baker, H2: *H. irrasa* Lane ex R.R. Sm., H3: *H. latispatha* Benth., H4: *H. mariae* Hook. f., H5: *H. mathiasiae G.S. Daniels & F.G. Stiles*, H6: *H. pogonantha* Cufod., H7: *H. wagneriana* Petersen, R1: *Renealmia alpinia* (Rottb.) Maas, R2: *R. cernua* (Sw. ex Roem. & Schult.) J.F. Macbr., R3: *R. pluriplicata* Maas.



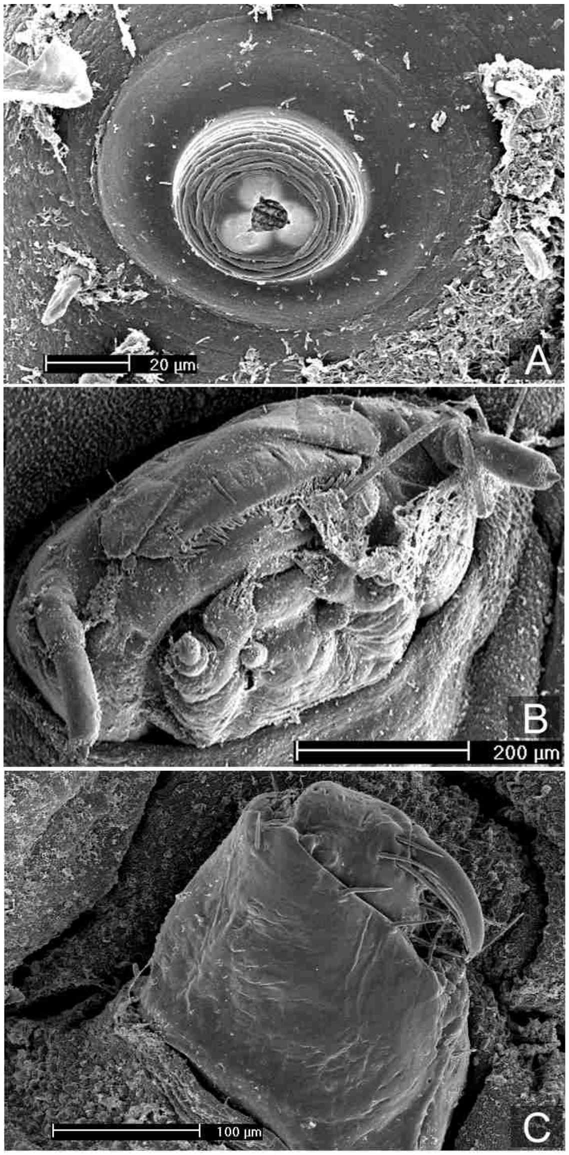
Figures 3.3. Larva of *Cephaloleia belti.* A. Dorsum longitudinal medial setose ridge. B. Spiracle. C. Head. D. Leg.



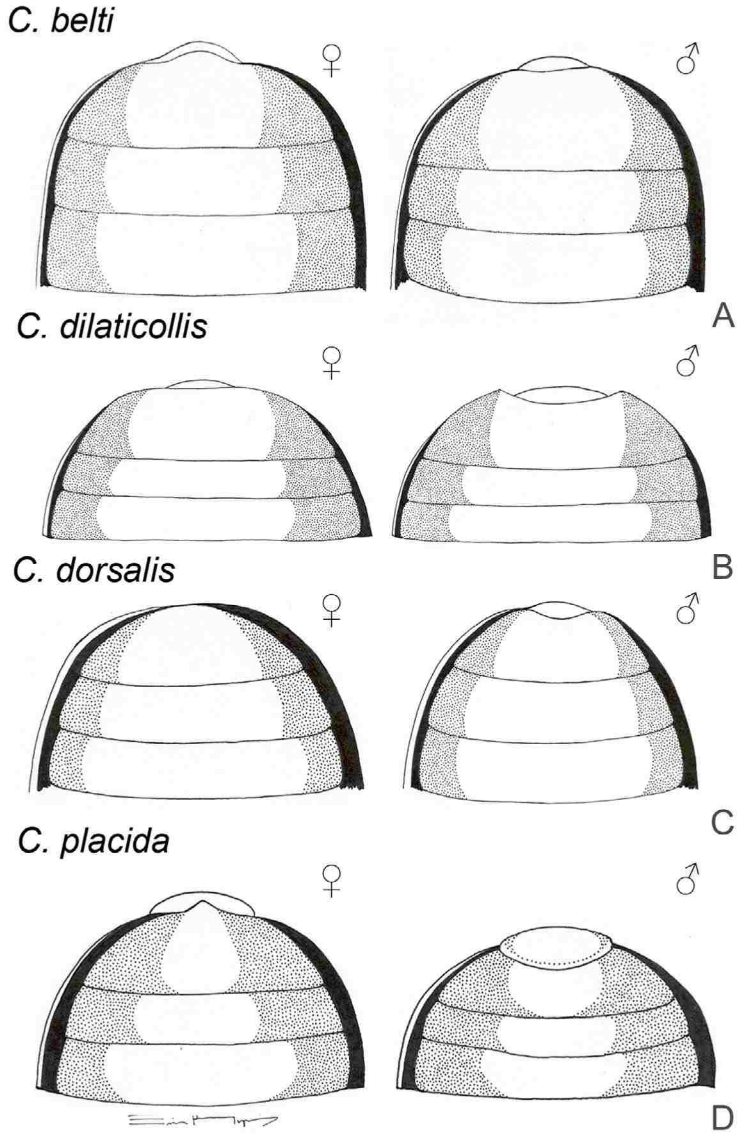
Figures 3.4. Larva of *Cephaloleia dilaticollis.* A. Spiracle. B. Head. C. Leg.



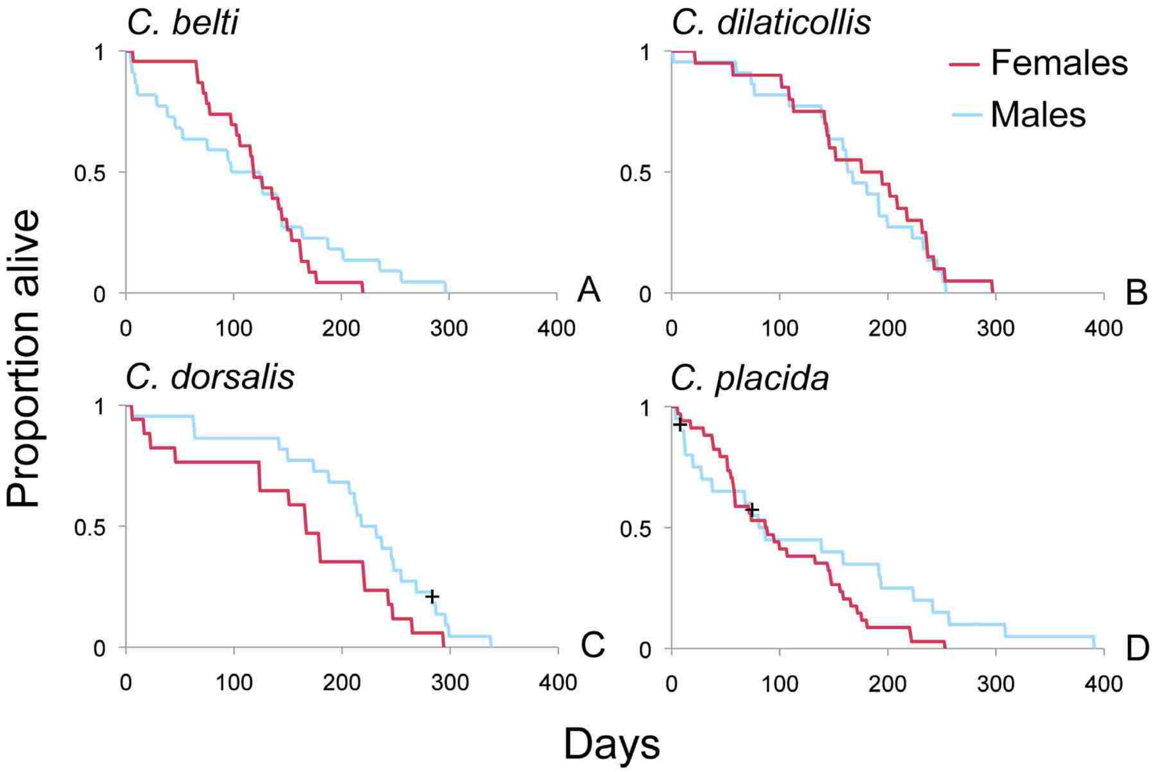
Figures 3.5. Larva of *Cephaloleia dorsalis.* A. Spiracle. B. Head. C. Leg.



Figures 3.6. Larva of *Cephaloleia placida.* A. Spiracle. B. Head. C. Leg.



Figures 3.7. Sexual dimorphism of the last sternite in four species of rolled-leaf beetles. A. *Cephaloleia belti.* B. *Cephaloleia dilaticollis.* C. *Cephaloleia dorsalis.* D. *Cephaloleia placida.* Illustration by E. K. Kuprewicz.



Figures 3.8. Survival of female and male adult *Cephaloleia* rolled-leaf beetles. A. *Cephaololeia belti*. B. *Cephaloleia dilaticollis*. C. *C. dorsalis*. D. *C. placida*. + Symbol represents censored observations. Host plants selected to feed *Cephaloleia* species: *C. belti* in *Heliconia latispatha* (Heliconiaceae), *C. dilaticollis* in *Renealmia alpinia* (Zingiberaceae), *C. dorsalis* in *Costus malortieanus* (Costaceae), *C. placida* in *Renealmia alpinia* (Zingiberaceae).

Chapter IV

Host plant scents attract rolled-leaf beetles to neotropical gingers in a Central American tropical rain forest[3]

SUMMARY

Leaf volatile chemicals are known to reduce herbivory rates by repelling or intoxicating insect herbivores and by attracting the predators and parasitoids of herbivores. However, leaf volatiles may also be used by insect herbivores as cues to locate their host plants. Leaf volatiles are suggested to be important host search cues for herbivores in structurally complex and diverse habitats, such as tropical rain forests. A group of insect herbivores, the rolled-leaf beetles (Coleoptera: Chrysomelidae: Hispinae), have maintained a highly specialized interaction with neotropical gingers (Zingiberales) for ca. 60 My. In this study, we explored chemical attraction to host plants under controlled laboratory conditions, using four sympatric rolled-leaf beetle species, *Cephaloleia dorsalis* Baly, *C. erichsonii* Baly, *C. fenestrata* Weise, and *C. placida* Baly. For each beetle species, we investigated (1) whether it was repelled or attracted by leaf scents produced by four host and four non-host plant species, including neotropical gingers in the families Marantaceae, Costaceae, and Zingiberaceae, and (2) its ability to use scents to detect its host plant. We found that rolled-leaf beetles can detect and are attracted by leaf volatiles from both host and non-host gingers.

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[3] García–Robledo, C. and C.C. Horvitz (2009). Host plant leaf scents attract rolled-leaf beetles to neotropical gingers (Zingiberales) in a Central American tropical rain forest. . Entomologia experimentalis et applicata.131: 115 -120

Additionally, when beetles were simultaneously exposed to leaf volatiles from host and non-host plants, three rolled-leaf beetle species were significantly more attracted by volatiles from their host plants than from non-hosts. Only one of the beetle species was not able to discriminate between host and non-host scents.

BACKGROUND

The release of volatile compounds from leaves is one of the mechanisms used by plants to reduce attack by insect herbivores. Primary functions of leaf volatiles are to directly repel or intoxicate insect herbivores, or to indirectly reduce herbivory rates by attracting predators and parasitoids of herbivores (De Moraes et al., 1998). The chemical composition of leaf volatiles varies among plant species. These species-specific blends of volatiles, although perhaps initially produced to reduce herbivory rates, are frequently used by insect herbivores as cues to identify and locate their host plants (Halitschke et al., 2008).

Leaf volatiles are suggested to be important host plant search cues for insect herbivores in structurally complex habitats with high plant species diversity, such as tropical rain forests (Bach, 1988). In the Neotropics, a group of herbivorous insects known as the rolled-leaf beetles (Coleoptera: Chrysomelidae: Hispinae) have maintained a highly specialized interaction with neotropical gingers (order Zingiberales) for ca. 60 My (Wilf et al., 2000; McKenna and Farrell, 2006; Gómez-Zurita et al., 2007; García-Robledo and Staines, 2008). Adult rolled-leaf beetles feed and mate within the scrolls formed by the young rolled leaves of their host plants. When the leaves expand and unfurl, adults must fly to another plant and colonize new young rolled leaves. The most speciose rolled-leaf beetle genus is *Cephaloleia*, with ca. 185 species (Staines, 1996).

This study focuses on four sympatric species of rolled-leaf beetles from the genus *Cephaloleia*, each with a diet restricted to plants from one family of Zingiberales. We performed this study at La Selva Biological Station, a tropical rain forest site located on the Atlantic slope of Costa Rica. At this site there are at least 40 sympatric species of *Cephaloleia* rolled-leaf beetles (Staines, 1996). Our aim was to determine, under laboratory conditions, whether leaf volatiles play a role in host plant detection and whether rolled-leaf beetles are able to discriminate between plant scents from their host plants compared with other Zingiberales.

STUDY AREA AND SPECIES OF INTEREST — This study was conducted in August-September 2005 and August-September 2006 at the Estación Biológica La Selva (hereafter La Selva), Puerto Viejo de Sarapiquí, Heredia Province, Costa Rica (10º26'N, 83º59'W). La Selva is classified as tropical wet forest (Holdridge, 1947) and receives an average of 4 000 mm rain per year (McDade et al., 1994). The La Selva reserve contains old-growth forest, secondary forest, swamp forest, and abandoned pasture land (McDade et al., 1994).

We selected four species of rolled-leaf beetles, each feeding only on members of one family of Zingiberales (Fig. 4.1). Host plants of these beetles are perennial herbs present in secondary forests at La Selva. When beetle species feed on plants of more than one species, we selected the most locally abundant host plant where both adults and larvae are most often found. At La Selva, *Cephaloleia dorsalis* Baly feeds on at least three species in the genus *Costus* (Costaceae). For this study, we selected *Costus malortieanus* Wendl. *Cephaloleia erichsonii* Baly feeds on at least five *Calathea* species (Marantaceae). For this study, we selected *Calathea gymnocarpa* H. Kenn. *Cephaloleia* *fenestrata* Weise feeds only on young rolled leaves of *Pleiostachya pruinosa* (W. Bull ex Regel) K. Schum. (Marantaceae) (Johnson, 2004). *Cephaloleia placida* Baly feeds on at least two native *Renealmia* species (Zingiberaceae) and is mostly found inside the scroll formed by the young rolled leaves of *Renealmia alpinia* (Rottb.) Maas.

METHODS

Individuals of *C. dorsalis, C. erichsonii, C. fenestrata*, and *C. placida* were collected in the field the same day that the choice trials were performed. Sex and mating status of the collected individuals were unknown. Beetles were brought to a laboratory maintained at 24-26 °C and they were deprived of food for 1 h before starting the trials.

Scent attraction experiments were performed in an olfactometer consisting of two lateral polystyrene boxes (17 × 15 × 5 cm; Fig. 4.2) that contained plant tissue from the plants to be tested. Polypropylene tubes (6 × 0.6 cm) connected the lateral boxes to a polyester-type 1 arena (27 × 1.5 cm). We punched 30 holes (0.2 mm in diameter) in the walls at the ends of the arena to allow the air to flow out of the olfactometer. Air was injected into the arena by an air pump connected to the lateral boxes at a rate of 10 ml/min. Leaf tissue used during the scent attraction trials was collected from unexpanded leaves. All leaves were rinsed with water, intending to remove any non-host plant scents that could potentially affect the laboratory trials. Fresh leaf tissue (300 cm^2) was placed inside the lateral boxes of the olfactometer.

Each trial consisted of placing an individual beetle in the arena through a central door (0.5 cm wide) that was thereafter sealed closed. After 10 min, we recorded the lateral box that was selected by the beetle. A choice was recorded only if the beetle had entered a connecting tube.

Sample sizes were variable because the goal was to have at least 10 choices by beetles and not all trials resulted in beetles making a choice. In total, there were 570 trials in experiment 1, resulting in 270 choices, and 454 trials in experiment 2 (238 choices). In experiment 1, the number of beetles making choices per plant-beetle combination ranged from 5 to 34, in experiment 2 this number ranged from 11 to 30. Each beetle was used in only one trial and then released. To avoid contamination of the olfactometer with beetle scents, the arena was discarded after each trial and replaced with a new one. To avoid contamination of the lateral boxes with scents from other plant species, each lateral box was rinsed with water and refilled only with leaves from the same plant species. To ensure that the lateral boxes constantly released leaf volatiles, plant samples were only used for two trials, then discarded.

The olfactometer was placed on a horizontal clean bench (Labconco©, USA). The room was ventilated after each trial by turning on the horizontal clean bench blower. All trials were performed in a windowless room and the position of the samples in the lateral boxes was alternated to avoid interference of light or spatial positioning of the olfactometer with the beetles' choices.

EXPERIMENT 1. ATTRACTION TO SCENTS FROM HOST PLANTS OR OTHER ZINGIBERALES — To determine whether rolled-leaf beetles detect leaf volatiles from their own host plants, we offered choices in the olfactometer between an empty lateral box and the scent from leaves of a host or non-host plant (Zingiberales) in the opposite lateral box. Beetle preferences were analyzed with a two-tailed binomial test.

EXPERIMENT 2. CHOICE BETWEEN SCENTS FROM HOST PLANTS OR OTHER ZINGIBERALES — To determine whether rolled-leaf beetles can discriminate between the scents from their own host or from non-host Zingiberales, we offered choices in the olfactometer between host and non-host leaf tissues in opposite lateral boxes. ‘Non-host’ refers to a member of the Zingiberales not included in a particular beetle’s diet, but which is a host of other rolled-leaf beetles included in this study. Beetle preferences were again analyzed with a two-tailed binomial test.

RESULTS

EXPERIMENT 1. ATTRACTION TO SCENTS FROM HOST PLANTS OR OTHER ZINGIBERALES — All four beetle species were attracted to their host plants when tested against an empty box in the olfactometer. Two beetles, *C. erichsonii* and *C. fenestrata*, were attracted to all plants offered (Fig. 4.3B, C), but the other two were not. Individuals of *C. dorsalis* were not attracted to the scent of *R. alpinia* (Zingiberaceae) (Fig. 4.3A), and *C. placida* were not attracted to *C. gymnocarpa* or *P. pruinosa* (Marantaceae) (Fig. 4.3D). None of the rolled-leaf beetle species were repelled by the four plant species tested (i.e., no preference for an empty box).

EXPERIMENT 2. CHOICE BETWEEN SCENTS FROM HOST PLANTS OR OTHER ZINGIBERALES — Choice experiments offering scents from host vs. non-host Zingiberales suggest differences between rolled-leaf beetle species. *Cephaloleia dorsalis*, *C. erichsonii*, and *C. placida* preferred the scents produced by their host plants (Fig. 4.4A, B, D), whereas *C. fenestrata* did not distinguish between host and non-host Zingiberales scents (Fig. 4.4C).

DISCUSSION

The behavior of rolled-leaf beetles in the laboratory suggests that they can detect – and are attracted to – scents from both host and several non-host plants. It is important to note that in all trials, we used sections of leaves. Leaf cutting is likely to induce green leaf volatiles, which are commonly released after mechanical damage (Dudareva et al., 2006). Green leaf volatiles are plant wound signals that are known to attract several chrysomelid species (Fernandez and Hilker, 2008). Therefore, it is possible that the general attraction of the four rolled-leaf beetle species to Zingiberales is based not only on leaf surface volatiles but also on green leaf volatiles. Although green leaf volatiles are usually not informative during the process of selection among host and non-host plants, in some chrysomelid species (e.g., *Cassida denticollis* Suffrian), the combination of green leaf and leaf surface volatiles has a synergistic effect, enhancing the herbivore's ability to differentiate between host and non-host plants (Müller and Hilker, 2000, Fernandez and Hilker, 2008).

Three of the four rolled-leaf beetle species included in this study were able to discriminate leaf scents of their own host plants from the scents of other Zingiberales. The use of leaf volatiles in host location is broadly documented for several chrysomelid genera, such as *Agelastica* (Park et al., 2004), *Diabrotica* (Hammack, 2001), *Leptinotarsa* (Bolter et al., 1997), *Oreina* (Kalberer et al., 2001), the flea beetles *Phyllotreta* (Pivnick et al., 1992), and the goldenrod leaf beetle *Trirhabda canadensis* (Kirby) (Puttick et al., 1988). Scents are assumed to be especially important for specialist herbivores such as the four species of rolled-leaf beetles included in this study (cf. Stenberg and Ericson, 2007). However, other stimuli may also play a role during host plant search in chrysomelids.

One species of rolled-leaf beetle included in this study, *C. fenestrata*, was unable to discriminate between scents from its host plant and scents from other Zingiberales.

This is unexpected, as this is the most specialized herbivore included in this study (i.e., it feeds only on one plant species of Marantaceae, viz., *P. pruinosa*). During host search, chrysomelids may use various stimuli in addition to scents, e.g., visual and contact cues (Heisswolf et al., 2007; Fernandez and Hilker, 2008). In some species, such as the flightless beetle *Altica engstroemi* Sahlberg, visual cues can be even more important than scent signals during host plant search (Stenberg and Ericson, 2007). Perhaps scents are useful for *C. fenestrata* only when combined with other signals such as visual cues. Other possibilities are that this beetle species does not respond to scents in the context of our laboratory setup, or that it does not use scents during host plant search.

In conclusion, rolled-leaf beetles have the ability to detect a range of scents from neotropical gingers. Some rolled-leaf beetle species can discriminate between host and non-host scents under laboratory conditions. Further research should elucidate how rolled-leaf beetles use the specific leaf scents to locate their hosts among the complex understory of the neotropical rain forest.

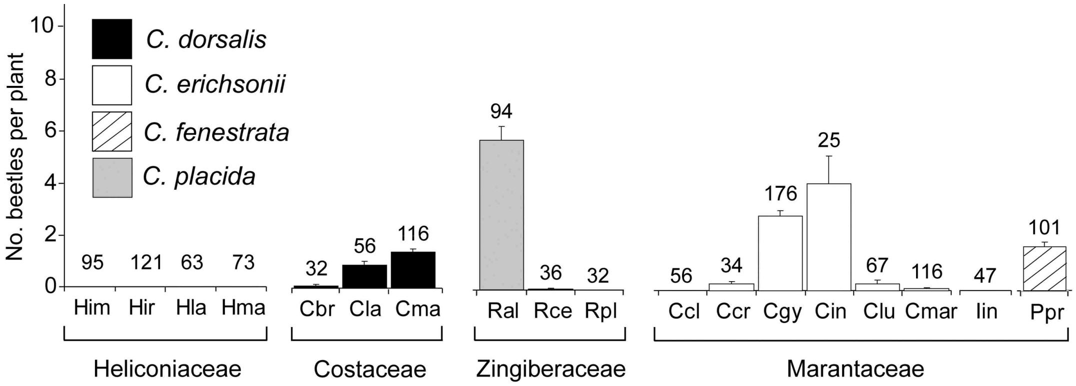

Figure 4.1. Density (mean number of beetle individuals per plant + SE) of the four rolled-leaf beetles included in this study on 18 host plant species of four families of the order Zingiberales. We recorded the number of rolled-leaf beetles present in rolled leaves at La Selva Biological Station (n = 1 340). Minimum distance between plants was 2 m. Numbers on the bars represent the number of plants checked. Family Heliconiaceae: Him, *Heliconia imbricata* (Kuntze) Baker; Hir, *H. irrasa* Lane ex R.R. Sm.; Hla, *H. latispatha* Benth.; Hma, *H. mathiasiae* G.S. Daniels & F.G. Stiles. Family Costaceae: Cbr, *Costus bracteatus* Gleason; Cla, *Costus laevis* Ruiz & Pav; Cma, *Costus malortieanus* H. Wendl.. Family Zingiberaceae: Ral, *Renealmia alpinia* (Rottb.) Maas; Rce, *R. cernua* (Sw. ex Roem. & Schult.) J.F. Macbr.; Rpl, *R. pluriplicata* Maas. Family Marantaceae: Ccl, *Calathea cleistantha* Standl.; Ccr, *C. crotalifera* S. Watson; Cgy, *C. gymnocarpa* H. Kenn.; Cin, *C. inocephala* (Kuntze) H. Kenn. & Nicolson; Clu: *C. lutea* Schult.; Cmar, *C. marantifolia* Standl.; Iin, *Ischnosiphon inflatus* L. Andersson; Ppr, *Pleiostachya pruinosa* (Regel) K. Schum.

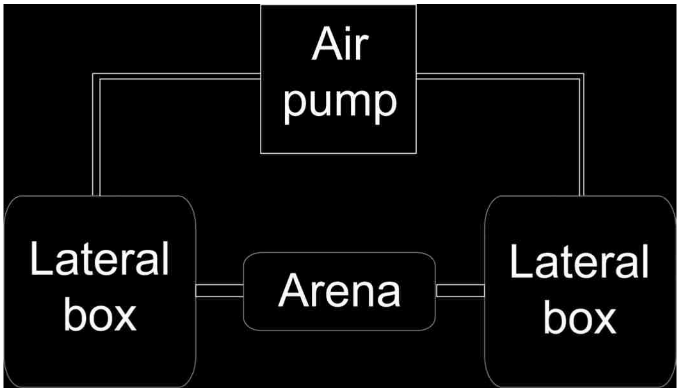

Figure 4.2. Diagram of the olfactometer used during the scent attraction experiments.

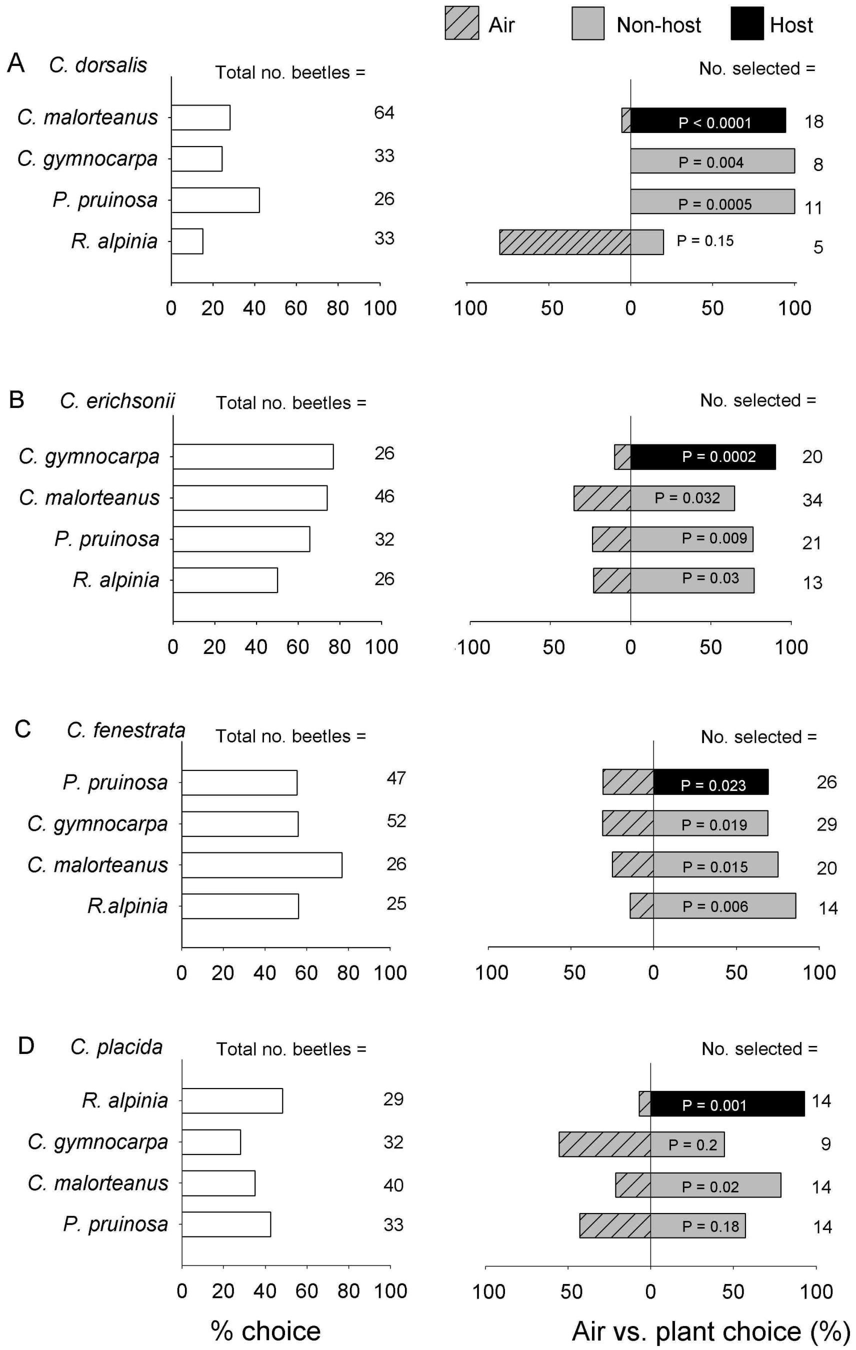

Figure 4.3. Percent of individuals of: A. *C. dorsalis*, B. *C. erichsonii*, C. *C. fenestrata*, and D. *C. placida* attracted to an empty box (air) or volatile chemicals from their host plant or other Zingiberales. Graphs in the left column represent the percent of individuals that made a choice. Graphs in the right column represent choices between an empty lateral box (air) and the plant alternatives. Numbers next to the bars represent sample sizes; P-values, two-tailed binomial test.

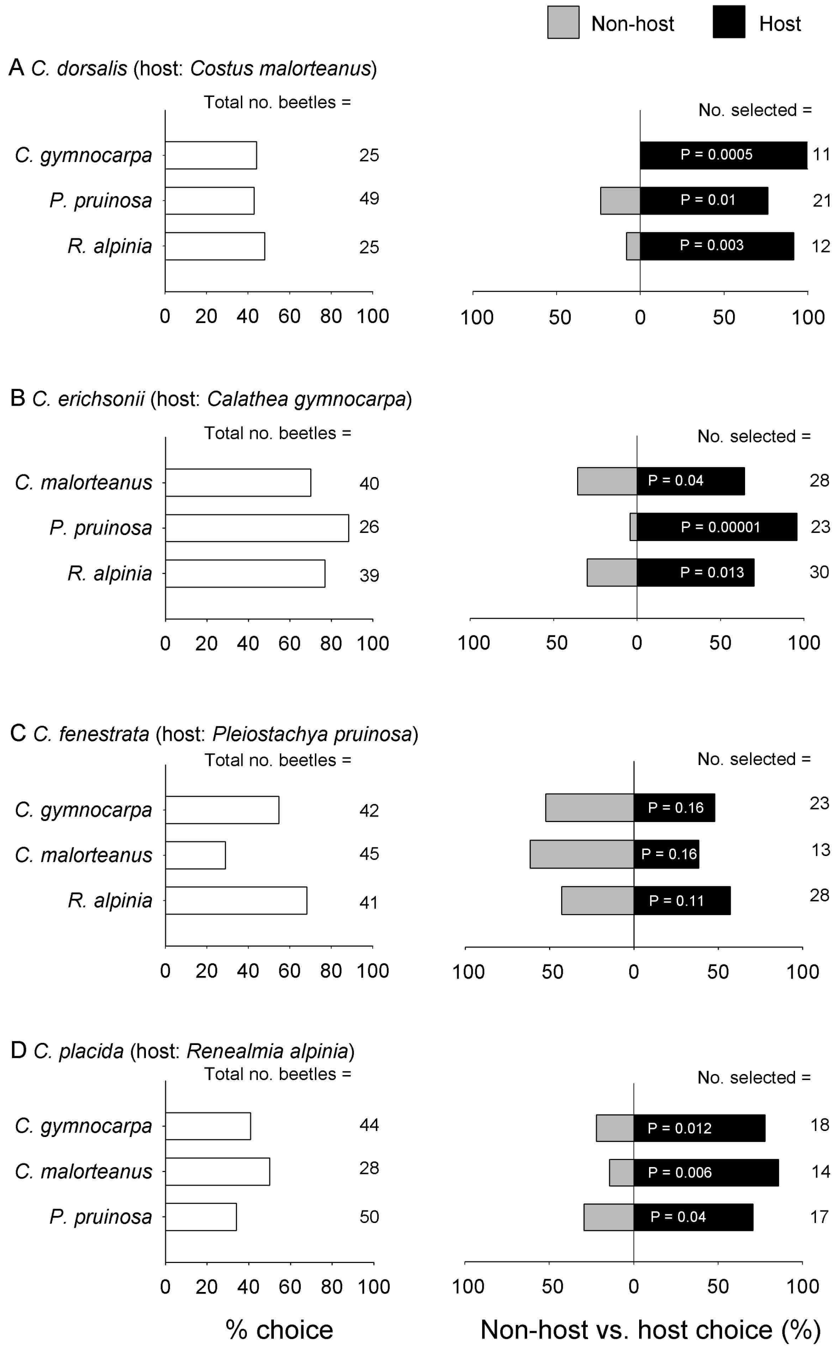

Figure 4.4. Percent of individuals of: A. *C. dorsalis,* B. *C. erichsonii*, C. *C. fenestrate*, and D. *C. placida* attracted to volatile chemicals from their host or other Zingiberales. Graphs in the left column represent the percent of individuals that made a choice. Graphs in the right column represent choices between host plants and other Zingiberales. Numbers next to the bars represent sample sizes; P-values, two-tailed binomial test.

Chapter V

Larval adaptation and adult ecological fitting regulate diet expansions to novel hosts in generalist and specialist rolled-leaf beetle herbivores[4]

SUMMARY

Novel plant-herbivore associations may be assembled by adaptation – an evolutionary process driven by natural selection, where organisms become better suited to their environments over generations, or by ecological fitting – an ecological process whereby herbivores colonize novel host plants as a result of the suites of preadapted traits that they carry at the time of colonization. Adaptation to novel host plants by insect herbivores may take several generations. In contrast, diet expansions through ecological fitting occur more quickly and without substantial evolutionary change. The association between neotropical gingers (order Zingiberales) and herbivores from the genus *Cephaloleia* (Coleoptera; Cassidinae) is one of the oldest and most conservative plant-herbivore interactions. At La Selva Biological Station, a tropical rain forest in Central America, two generalist and two specialist *Cephaloleia* species are expanding their diets to exotic gingers from South America and the Paleotropics. This study compared the roles of adaptation and ecological fitting for generalist and specialist *Cephaloleia.* We evaluated both preference for and performance on historical and novel host plants at both larval and adult stages. We found that a change in diet by larvae would require adaptation to the novel hosts, while adults were preadapted. The main conclusion is that both adaptation and ecological fitting are playing fundamental roles in diet expansions in *Cephaloleia* beetles.

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BACKGROUND

Insect herbivores represent an important component of terrestrial organic diversity. It is estimated that 25 percent of the described species on earth are phytophagous insects (Mayhew 2001). One of the processes generating this outstanding diversity is the interaction between plants and their insect herbivores (Ehrlich and Raven 1964). Over generations, plants and insects are expected to coevolve by reciprocally adapting to changes in traits that affect herbivore host plant use and plant defenses against herbivores (Janzen 1980; Futuyma and Slatkin 1983).

It is clear that reciprocal adaptation plays a major role during the diversification of plants and their associated insect herbivores (Agrawal 2007). However, phylogenies of insect herbivores usually are more concordant with host plant phylogenies at higher than at lower taxonomic levels (Funk et al. 1995). One explanation for this pattern is that the likelihood of diet expansions to novel host plants increases as plants are more taxonomically related. This is a consequence of phylogenetic conservatism, where chemical and morphological similarities increase as plant species are more closely related (Futuyma and Mitter 1996).

During diet expansions in low taxonomic levels, it is more likely that herbivore genotypes are preadapted to the novel host plants (Agosta and Klemens 2008). It is suggested that in this scenario, the process assembling novel plant-herbivore associations may not result from adaptation but from ecological fitting – the process whereby organisms colonize novel habitats and form novel associations as a result of the suites of traits that they carry at the time they encounter the novel condition (Agosta 2006; Agosta and Klemens 2008).

Diet expansions assembled by ecological fitting must fulfill a complex series of requirements. First, they entail the simultaneous pre-adaptation of herbivore behavioral and physiological traits involved in both host plant preferences and performance. Second, it is required that the traits of both larval and adult stages must be pre-adapted for successful colonization of novel hosts.

Another factor potentially affecting the probability of diet expansion to novel host plants is the diet breadth of insect herbivores. Although diets of most insect herbivores are highly specialized, some insect species feed on a broad range of plant species. If broader diet breadths are associated with a higher diversity or plasticity in traits involved in host use, it is reasonable to expect that the likelihood of ecological fitting is higher for generalist than specialist herbivores.

One of the oldest and most conservative plant-herbivore associations is the interaction between neotropical gingers (Order Zingiberales) and beetles of the neotropical genus *Cephaloleia* (Chrysomelidae, Cassidinae) (Wilf et al. 2000; McKenna and Farrell 2005; McKenna and Farrell 2006; García-Robledo and Staines 2008). *Cephaloleia* beetles are also known as the “rolled-leaf beetles” because the adults of most species feed and mate inside the scrolls formed by the young rolled leaves of their host plants (Strong 1977). It is estimated that *Cephaloleia* beetles and neotropical Zingiberales have interacted for the last 40 – 60 MY in isolation from paleotropical Zingiberales (Wilf et al. 2000; McKenna and Farrell 2006; García-Robledo and Staines 2008).

At La Selva Biological Station (Costa Rica, Central America), the tropical rain forest were we performed this research, at least 40 species of *Cephaloleia* associated with at least 43 native plants from the order Zingiberales (Staines 1996) are present. In the last decade five paleotropical and one South American species of exotic Zingiberales have been found at the La Selva Biological Station. Seven *Cephaloleia* species with contrasting diet breadths are currently expanding their diets to the exotic Zingiberales, generating 16 novel plant-herbivore interactions (Chapter I).

These incipient diet expansions to exotic Zingiberales by *Cephaloleia* beetles represent an opportunity to understand the processes involved in the establishment of novel plant-herbivore interactions. Here we explore the role of adaptation and ecological fitting during diet expansions to novel hosts. We selected two generalist and two specialist *Cephaloleia* beetles, focusing on two main components of diet expansion in larval and adult stages, feeding preference and survival.

In this study we explore the relative preference and performance of larvae and adult *Cephaloleia* beetles on native and novel hosts. If larval and adult preference and survival are higher on the native than the novel host plants, this suggests that evolutionary changes will be required for the populations on the novel host to attain equivalent fitness to that attained on the native host. This scenario would support adaptation as the process by which novel plant-herbivore interactions are assembled. It is also possible that preference and survival are preadapted to the exotic plants, such that preference and survival are equal for the native and novel hosts, or even higher for the novel hosts. This scenario would support ecological fitting as the process by which novel plant-herbivore interactions between *Cephaloleia* beetles and exotic Zingiberales are assembled.

The objectives of this research are: 1. To determine if generalist and specialist *Cephaloleia* beetles differ in their patterns of preference and performance for native and novel host plants. 2. To determine if larval and adult stages of *Cephaloleia* beetles expanding their diets to novel hosts display: a. pre-adapted host preference and survival for the novel host plants and/or b. need to adapt to the exotic Zingiberales to attain equivalent preference and survival for native and novel hosts.

METHODS

STUDY SITE AND SPECIES — This study was conducted from August 2005 to March 2009 at La Selva Biological Station (hereafter La Selva), a tropical rain forest in Costa Rica, Central America (10º26’N, 83º59’W). We selected four *Cephaloleia* beetle species with contrasting diet breadths as study species (Table 5.1). At La Selva, *Cephaloleia belti* is the species with the broadest diet breadth, feeding on 15 species from three families of Zingiberales (García-Robledo et al. in press). The beetle *Cephaloleia dilaticollis* is also a generalist, feeding on ten species from three families of Zingiberales (García-Robledo et al. in press). We also selected two species with specialized diets. *Cephaloleia dorsalis* feeds on four species in the family Costaceae and *Cephaloleia placida* feeds on two species in the family Zingiberaceae (García-Robledo et al. in press).

Adults of the four *Cephaloleia* species feed on the leaf tissue of the young rolled leaves of their host plants. In contrast, larvae feed on the leaf tissue of expanded leaves (García-Robledo et al. in press). At La Selva, the four beetle species are currently expanding their diets to exotic hosts from India, the Malay Peninsula, the Pacific Islands and South America (Table 5.1).

LARVAE ACCEPTABILITY AND SURVIVAL IN NATIVE AND NOVEL HOST PLANTS — Larvae of *Cephaloleia* remain in the same host plant through their development. Therefore in nature larvae of *Cephaloleia* don't have to experience situations where they have to select between different host plants. For this reason, we tested for differences in acceptability (estimated as differences in feeding rates in a non-choice setup) between native and novel hosts. We also estimated differences in survival of *Cephaloleia* larvae reared in native or novel host plants. To estimade the acceptability of native and novel hosts, we collected males and females of each *Cephaloleia* species from the native species of hosts on which larvae and adults of each beetle species were most frequently found (García-Robledo *et al.* in press; Table 5.1). Mating couples were placed in separate 17 X 15 X 5 cm containers and fed *ad libitum* with leaf tissue from their native host plants (Number of matin couples: $N_{C.belti}$ = 38, $N_{C.dilaticollis}$ = 32, $N_{C.dorsalis}$ = 37, $N_{C.placida}$ = 42). Eggs were collected and after eclosion, larvae were randomly assigned to one of the following diets: leaf tissue from the native host or leaf tissue from one of the novel host plant (Table 5.1). Each larva was placed in an individual container and fed with two 3.5 cm diameter disks of leaf tissue. Larvae were reared at a mean temperature of 27°C and a light regime of 12 h light: 12 h (sample size in Table 5.2).

Differences in acceptability between native and novel hosts were estimated by measuring the area of tissue consumed by each larva 48 h after larval eclosion. Leaf area consumed was measured using a grid divided in 1 X 1 mm squares. When there were only two host plants to compare (i.e. one native and one novel host plant), differences in area consumed between native and novel host plants were determined by using Welch

Two Sample t-tests. When there were three host plants to compare, we used one-way ANOVAs.

To estimate larval survival in native and novel host plants, we fed and monitored each larva every 48 h until death or pupation. Differences in larval mortality between native and novel hosts were determined by Kaplan-Meyer survival analyses.

ADULT PREFERENCE AND SURVIVAL IN NATIVE AND NOVEL HOST PLANTS — Preferences and survival of adult insects can be potentially affected by their feeding experience as a larva, or by their gender (Mevi-Schutz and Erhardt 2003). For this reason, in the following experiment we include both diet as a larva and gender as factors affecting adult preference and survival.

To determine the feeding preferences of adult *Cephaloleia* beetles for native or novel hosts (estimated as differences in feeding rates in a choice setup), we reared larvae of the four species of *Cephaloleia* beetles on both the native and novel hosts (Table 5.1). Pupae were placed in individual containers. We determined the gender of each adult that emerged. For each beetle species we simultaneously offered to each individual 3.5 cm diameter discs of leaf tissue from the native host and the novel host plants. For the specialist *Cephaloleia dorsalis* each choice trial consisted in offering leaf tissue from the native host *C. malortieanus* and the novel host *Ch. speciosus.* For the other four beetle species, we simultaneously offered leaf tissue from their native host and two novel host plants (Table 5.1). Individual beetles were only used in one one trial. After 12 h we measured the leaf area consumed using a grid divided in 1 X 1 mm squares. To test for differences in leaf tissue consumed in native and novel host plants, for each beetle species we performed a two-way ANOVA where each adult feeding on native and novel hosts were treated as a block. Diet as a larva and gender were included as fixed factors (*see* sample size in Table 5.3)**.**

To determine differences in survival of adults feeding on native or novel hosts, we obtained adults of each beetle species from larvae reared on native or novel hosts (Table 5.1). The gender of each adult was recorded before being placed in individual containers. Adults were fed *ad libitum* with leaf tissue of either their native or their novel host plant (Table 5.1). Time to death was recorded by monitoring each beetle every 48 h for 429 days, by which date all of the beetles had died. We explored differences in adult survival among diets with a fully crossed ANOVA design that included diet as larva, diet as adult and gender as fixed factors. The response variable was the square root-transformed time to death (*see* sample size in Table 5.4).

RESULTS

LARVAL ACCEPTABILITY AND SURVIVAL IN NATIVE AND NOVEL HOST PLANTS

— In general, larvae consumed more leaf tissue of native hosts than of novel hosts and larval survival was higher in the native than the novel host plants. Newborn larvae of the generalist beetle *C. belti* consumed 16 to 27 % more leaf tissue from the native host, *H. latispatha* than from the novel hosts *H. psittacorum* and *M. velutina* (F_2 = 13.55, P < 0.0001, Figure 5.1A, Table 5.2). Larval survival of *C. belti* was 16 – 23% higher in the native than in the novel hosts (χ^2 = 44.82, DF = 2, P < 0.001 Figure 5.1E, Table 5.2).

Larvae of the generalist beetle *C. dilaticollis* consumed two times more leaf tissue from its native host *R. alpinia* and the novel host *A. purpurata* than from the novel host

H. coronarium ($F_2 = 21.41$, $P < 0.0001$, Figure 5.1B, Table 5.2). Larval survival of *C. dilaticollis* was 11 -1 5% higher in the native host *R. alpinia* and the novel host *A. purpurata* than on the novel host *H. coronarium* ($\chi^2 = 26.02$, DF = 2 , $P < 0.001$ Figure 5.1E, Table 5.2).

The feeding rates of larva reared on the native host *C. malortieanus* were 21% higher in the native host *C. malortieanus* than the novel host plant *Ch. speciosus* (Welch Two Sample t-test, DF = 646.9, $t = 3.54$, $P = 0.0004$, Figure 5.1C, Table 5.2). Larval survival of *C. dorsalis* was 10% higher in the native host *C. malortieanus* than in the novel host *Ch. speciosus* ($Z = 3.44$, DF = 1, $P < 0.001$ Figure 5.1G, Table 5.2).

Feeding rates of larvae of the specialist beetle *C. placida* reared in the native host *R. alpinia* were 35 – 67 % higher than in the novel hosts *A. purpurata* and *H. coronarium* ($F_2 = 46.39$, $P < 0.0001$, Figure 5.1D, Table 5.2). Larval survival of *C. placida* was higher in the native host *R. alpinia* than in the novel host *A. purpurata* and *H. coronarium* ($\chi^2 = 28.78$, DF = 2 , $P < 0.001$ Figure 5.1H, Table 5.2).

EFFECTS OF LARVAL DIET ON ADULT PREFERENCE AND LONGEVITY — Host selection and survival of adults reared in native or novel hosts were not affected by their feeding experience as a larva. For the generalist species *C. belti,* we were able to obtain adults from larvae reared on the native host *H. latispatha,* as well as from larvae reared on the novel hosts *H. psittacorum* and *M. velutina.* In *C. belti* adult preferences were not affected by the diet experienced during larval stages ($F_{2,467} = 0.99$, $P = 0.37$, *see* sample size in Table 5.3) or longevity ($F_2 = 1.56$, $P = 0.21$, *see* sample size in Table 5.4) in native and novel.

For the generalist species *C. dilaticollis,* mortality of larvae reared on one of the two novel hosts, *H. coronarium,* was exceptionally high (Mortality = 87.4%, Table 5.2). For this treatment, very few adults were available. For this reason we restricted our studies of the effect of larval diet on adult preference and longevity to larvae reared on the native host *R. alpinia* and *A. purpurata*, for which we did obtain sufficient adults. Adult host choice preferences ($F_{1, 185} = 0.56$, $P = 0.45$, *see* sample size in Table 5.3) and longevity ($F_{1} = 0.0.38$, $P = 0.54$, *see* sample size in Table 5.4) was not affected by the diet of the beetles during the larval stage.

For the specialist species *C. dorsalis,* we were able to obtain adults from larvae reared on both the native host *C. malortieanus* and the novel host *Ch. speciosus.* Host plant choice ($F_{1, 95} = 3.74$, $P = 0.06$, *see* sample size in Table 5.3) and longevity ($F_{1} = 0.18$, $P = 0.67$, *see* sample size in Table 5.4) of adult beetles in native and novel hosts are not affected for the diet as larvae in this beetle species.

For the specialist beetle species *C. placida,* we obtained sufficient adults only from larvae raised on the native host *R. alpinia.* Mortality of larvae reared on both novel hosts *A. purpurata* and *H. coronarium* was exceptionally high (mortality in *A. purpurata* = 85.9%, mortality in *H. coronarium* = 96.7%, Table 5.2). Therefore, for this species we were not able to analize the effects of larval diet on adult preference and longevity.

EFFECT OF GENDER ON ADULT PREFERENCE AND LONGEVITY — For all species, males and females made the same choices in host preference trials. However, specialist males lived longer than females.

Males and females of the generalist species *C. belti* and *C. dilaticollis* displayed similar host preferences (*C. belti*: $F_{1, 467} = 0.85$, $P = 0.36$, *C. dilaticollis:* $F_{1, 185} = 0.068$,

$P = 0.79$, Table 5.3). Generalist males and females lived as long on native as they did on novel hosts(*C. belti*: $F_1 = 0.35$, $P = 0.55$, *C. dilaticollis:* $F_1 = 0.58$, $P = 0.44$, Table 5.4).

Males and females of the specialist species *C. dorsalis* and *C. placida* did not differ in host choice preferences (*C. dorsalis*: $F_{1, 95} = 0.45$, $P = 0.50$, *C. placida:* $F_{1, 36} = 0.17$, $P = 0.68$, Table 5.3). However, males of these species lived longer than females (*C. dorsalis*: $F_1 = 10.28$, $P = 0.002$, Mean ± SD $_{\text{females}} = 162 \pm 83.72$ d., Mean ± SD $_{\text{males}} = 223.58 \pm 97.71$ d. *C. placida:* $F_1 = 4.05$, $P = 0.04$, Mean ± SD $_{\text{females}} = 78.76 \pm 64.26$ d. , Mean ± SD $_{\text{males}} = 98.79 \pm 114.31$ d., Table 5.4).

ADULT PREFERENCE AND LONGEVITY IN NATIVE AND NOVEL HOST PLANTS — In general, adults display similar preference and longevity in both native and novel hosts. Adults may even prefer and live longer in the novel than in the native host plants.

Adults of the generalist species *C. belti* preferred to feed on their novel hosts ($F_{2, 904} = 9.12$, $P = 0.0001$, Figure 5.2A, Table 5.3). Adults of *Cephaloleia belti* lived longer on the novel host *H. psittacorum* than on the native host *H. latispatha* and the novel host *M. velutina* ($F_2 = 20.53$, $P < 0.0001$, Figure 5.2E, Table 5.4).

Adults of the generalist beetle *C. dilaticollis* preferred to feed on the novel host *A. purpurata* over the native host *R. alpinia* and the novel host *H. coronarium* ($F_{2, 185} = 12.38$, $P < 0.0001$, Figure 2B, Table 5.3). *Cephaloleia dilaticollis* lived longer in the novel host *A. purpurata* than in the native host *R. alpinia.* Adults of *C. dilaticollis* lived shorter when feeding on the novel host *H. coronarium* ($F_2 = 28.72$, $P < 0.0001$, Figure 5.2F, Table 5.4).

Adults of the specialist beetle *C. dorsalis* preferred to feed on the novel host *Ch. speciosus* over the novel host *C. malortieanus* ($F_{1, 64} = 111.68$, $P < 0.0001$, Figure 5.2C, Table 5.3). Adult survival was equivalent in both host plants ($F_1 = 0.08$, $P = 0.77$, Figure 5.2G, Table 5.4)**.**

Adults of the specialist beetle species *C. placida* preferred to feed on its native host plant *R. alpinia* and the novel host *A. purpurata* over the novel host *H. coronarium* ($F_{2, 45} = 7.00$, $P < 0.002$, Figure 5.2D, Table 5.3). Adult longevity was higher in the native host *R. alpinia* and the novel host *A. purpurata* than in the novel host *H. coronarium* ($F_2 = 57.31$, $P < 0.0001$, Figure 5.2H, Table 5.4).

DISCUSSION

In this study, contrary to expectations,we did not find that generalists were more preadapted to diet expansions than specialists. However, in all species we observed that larval larval stages preferred and performed better in native hosts, while adult stages prefer to feed on the novel host plants, where they display higher longevity. These results suggest different levels of specialization between larval and adult stages of *Cephaloleia* beetles. Larval stages are apparently more specialized in their native host plants than adults. Conversely, adult diets are generalized to the extent of being preadapted to host plants that they have never interacted with previously.

The patterns of specialization and generalization at different developmental stages reported in this study are also relevant to our current understanding of novel interactions between exotic plants and native herbivores. One of the explanations for the success of some exotic plants in new habitats is the Enemy Release Hypothesis (Keane and Crawley

2002; Verhoeven et al. 2009). This hypothesis predicts that exotic plants should escape herbivore attacks in invaded habitats because native herbivore preferences and physiology would not be adapted to the novel hosts (Keane and Crawley 2002). Our results reveal that preference and performance of native herbivores on exotic hosts can vary between developmental stages. Although larval stages of *Cephaloleia* beetles may feed less and survive poorly on the novel hosts, as predicted by the enemy release hypothesis, adults do not coose native over novel hosts and survive equall well on novel hosts as they do on native hosts.

Our results raise two questions: 1. Why are the diets of larval stages of *Cephaloleia* beetles more restricted to the native hosts? and 2. Why are adult diets of *Cephaloleia* beetles preadapted to the novel host plants? Larvae of *Cephaloleia* beetles feeding on the native hosts are highly efficient at acquiring nutrients, however they display narrow physiological tolerance to slight changes in the chemical composition of host plant leaf tissue (Auerbach and Strong 1981). If there is a cost of adaptation, represented as a trade-off between host use efficiency and diet breadth (as predicted by the “Jack of all Trades Master of None Principle”, Futuyma and Moreno 1988), it is possible that adaptations that lead to higher performance in native hosts preclude an efficient use of novel host plants.

Larvae of *Cephaloleia* beetles have a limited mobility, developing on the same individual where eggs were laid by mothers. In contrast, adults are highly mobile, dispersing to new plants several times during their lifetimes in search of rolled leaves (Johnson 2004a, b, 2005). Rolled leaves are an ephemeral resource; once the leaves unfurl fully they are no longer a suitable habitat. Therefore, adults play a major role in encountering and colonizing new resources. It is conceivable that adult *Cephaloleia* beetles were selected to maintain the generalized detoxification systems required for colonizing potential new hosts. The increased adult longevity observed in most of the interactions with novel hosts is an unexpected finding. It is possible that native plant chemical defenses and nutritional characteristics are adapted to reduce the potential longevity of native herbivores. If the defenses of exotic Zingiberales, that evolved under the selection of old-world insect herbivores have a reduced toxicity against *Cephaloleia* beetles , the realized longevity of adult *Cephaloleia* beetles is expected higher in the novel hosts than in the native hosts. An increased longevity in adult insects exposed to novel diets was also observed in laboratory experiments with med flies (*Ceratitis capitata*). The reason for increased longevity in novel environments remains unknown (J. Carey 2010, personal communication).

In the diet expansions to exotic plants reported in this research, native herbivores faced parent-offspring conflicts. Parents and offspring *Cephaloleia* beetles displayed different behavioral preferences and opposite performance in the native and novel host plants. Similar parent-offspring conflicts are reported for other insect herbivores (Scheirs et al. 2000). In insects where larvae and adults feed on different resources, such as Lepidoptera, females frequently oviposit in host plants that are suboptimal hosts for their offspring (Thompson 1988). Selection does not eliminate this apparently maladaptive behavior over generations (Thompson 1988). In insects where both immature and adult stages feed on the same resource, oviposition preferences usually match host plants that increase the survival of adults, not of their offspring (Chew 1977; Scheirs et al. 2000; Mayhew 2001).

Although we did not record oviposition preferences for native or exotic hosts in this study, it is likely that higher feeding preferences coupled with higher longevity of adults in the exotic hosts increase the probability of oviposition in the novel host plants. In the forest, adults of *Cephaloleia belti* aggregate and mate in the host plant with the highest rank of preference (García-Robledo unpubl. data). If adult preference and increased longevity in the novel hosts are positively correlated with oviposition, our results support the following diet expansion scenario. Preadapted adult beetles promote the colonization of novel hosts where their offspring display a low performance. If genetic variation is available within the populations, we may expect over generations natural selection to favor either a reduction of adult preferences for the exotic plants, or larvae with higher performance on the novel hosts.

The assemblage of novel plant-herbivore interactions through adaptation is assumed to take several generations (Keeler and Chew 2008). In contrast, diet expansions through ecological fitting are assumed to occur more quickly and without substantial evolutionary change because herbivores are preadapted to the novel hosts (Thomas et al. 1987; Agosta and Klemens 2008). The main conclusion of this study is that both adaptation and ecological fitting are playing fundamental roles in diet expansions in *Cephaloleia* beetles, generating parent-offspring conflicts. The implication is that diet expansions in *Cephaloleia* beetles entail a complex process of optimization of tradeoffs of adult fitness through an increase in adult longevity, or increasing their fitness through the survival of their offspring in native and novel hosts.

Table 5.1. *Cephaloleia* beetles and the native and exotic host plants selected for preference and survival experiments.

Beetle species	Native host plant	Novel host plants	Origin of novel hosts
Cephaloleia belti (Generalist)			
	Heliconia latispatha (Heliconiaceae)	*Heliconia psittacorum* (Heliconiaceae)	Caribbean, northern South America
		Musa velutina (Musaceae)	India
Cephaloleia dilaticollis (Generalist)			
	Renealmia alpinia (Zingiberaceae)	*Alpinia purpurata* (Zingiberaceae)	Pacific islands
		Hedychium coronarium (Zingiberaceae)	Eastern India
Cephaloleia dorsalis (Specialist)			
	Costus malorteanus (Costaceae)	*Cheilocostus speciosus* (Costaceae)	Malay Peninsula of Southeast Asia
Cephaloleia placida (Specialist)			
	Renealmia alpinia (Zingiberaceae)	*Alpinia purpurata* (Zingiberaceae)	Pacific islands
		Hedychium coronarium (Zingiberaceae)	Eastern India

Table 5.2. Sample sizes for experiments testing differences in acceptability and survival in larvae of *Cephaloleia* reared in native and novel host plants.

		Number of larvae	
Herbivore species	Diet	Acceptability trials	Survival trials
Cephaloleia belti (38$_a$)			
	H. latispatha (Native)	334	348
	H. psittacorum (Novel)	374	389
	M. velutina (Novel)	391	397
Cephaloleia dilaticollis (32$_a$)			
	R. alpinia (Native)	545	574
	A. purpurata (Novel)	526	541
	H. coronarium Novel)	184	213
Cephaloleia dorsalis (37$_a$)			
	C. malortieanus (Native)	337	340
	Ch. speciosus (Novel)	316	323
Cephaloleia placida (42$_a$)			
	R. alpinia (Native)	268	272
	A. purpurata (Novel)	214	220
	H. coronarium (Novel)	239	240

$_a$ Number of females collected in the field from which larvae were obtained for acceptability and survival trials.

Table 5.3. Sample sizes for experiments testing differences in feeding preferences between native and novel hosts for male and female adults of *Cephaloleia*. Adults were were obtained from larvae reared in either native or novel host plants.

		Number of adults	
Herbivore species	Diet as a larva	Females	Males
Cephaloleia belti			
	H. latispatha (Native)	96	103
	H. psittacorum (Novel)	82	81
	M. velutina (Novel)	77	68
Cephaloleia dilaticollis			
	R. alpinia (Native)	32	25
	A. purpurata (Novel)	26	35
	H. coronarium (Novel)	--[a]	--[a]
Cephaloleia dorsalis			
	C. malortieanus (Native)	39	42
	Ch. speciosus (Novel)	20	30
Cephaloleia placida			
	R. alpinia (Native)	32	35
	A. purpurata (Novel)	--[a]	--[a]
	H. coronarium (Novel)	--[a]	--[a]

[a] Trials not performed because larval mortality was high, precluding obtaining the required adults for preference trials.

Table 5.4. Sample sizes for experiments testing differences in longevity in female and male adults of *Cephaloleia* feeding on native or novel hosts. Adults were obtained from larvae reared in either native or novel host plants.

			Number of adults	
Beetle species	Diet as a Larva	Diet as adult	Females	Males
Cephaloleia belti				
	H. latispatha (Native)			
		H. latispatha (Native)	23	22
		H. psittacorum (Novel)	18	22
		M. velutina (Novel)	20	22
	H. psittacorum (Novel)			
		H. latispatha (Native)	17	21
		H. psittacorum (Novel)	17	18
		M. velutina (Novel)	18	22
	M. velutina (Novel)			
		H. latispatha (Native)	18	18
		H. psittacorum (Novel)	17	18
		M. velutina (Novel)	20	19
Cephaloleia dilaticollis				
	R. alpinia (Native)			
		R. alpinia (Native)	20	22
		A. purpurata (Novel)	20	14
		H. coronarium (Novel)	20	21
	A. purpurata (Novel)			
		R. alpinia (Native)	13	14
		A. purpurata (Novel)	14	14
		H. coronarium (Novel)	13	16
	H. coronarium (Novel)			
		R. alpinia (Native)	--[a]	--[a]
		A. purpurata (Novel)	--[a]	--[a]
		H. coronarium (Novel)	--[a]	--[a]

[a] Trials not performed because larval mortality was high, precluding obtaining the required adults for longevity trials.

Table 5.4. (Contd.)

			Number of adults	
Beetle species	Diet as a Larva	Diet as adult	Females	Males
Cephaloleia dorsalis				
	C. malortieanus (Native)			
		C. malortieanus (Native)	17	21
		Ch. speciosus (Novel)	16	20
	Ch. speciosus (Novel)			
		C. malortieanus (Native)	24	14
		Ch. speciosus (Novel)	16	20
Cephaloleia placida				
	R. alpinia (Native)			
		R. alpinia (Native)	34	17
		A. purpurata (Novel)	35	19
		H. coronarium (Novel)	19	21
	A. purpurata (Novel)			
		R. alpinia (Native)	--[a]	--[a]
		A. purpurata (Novel)	--[a]	--[a]
		H. coronarium (Novel)	--[a]	--[a]
	H. coronarium (Novel)			
		R. alpinia (Native)	--[a]	--[a]
		A. purpurata (Novel)	--[a]	--[a]
		H. coronarium (Novel)	--[a]	--[a]

[a] Trials not performed because larval mortality was high, precluding obtaining the required adults for longevity trials.

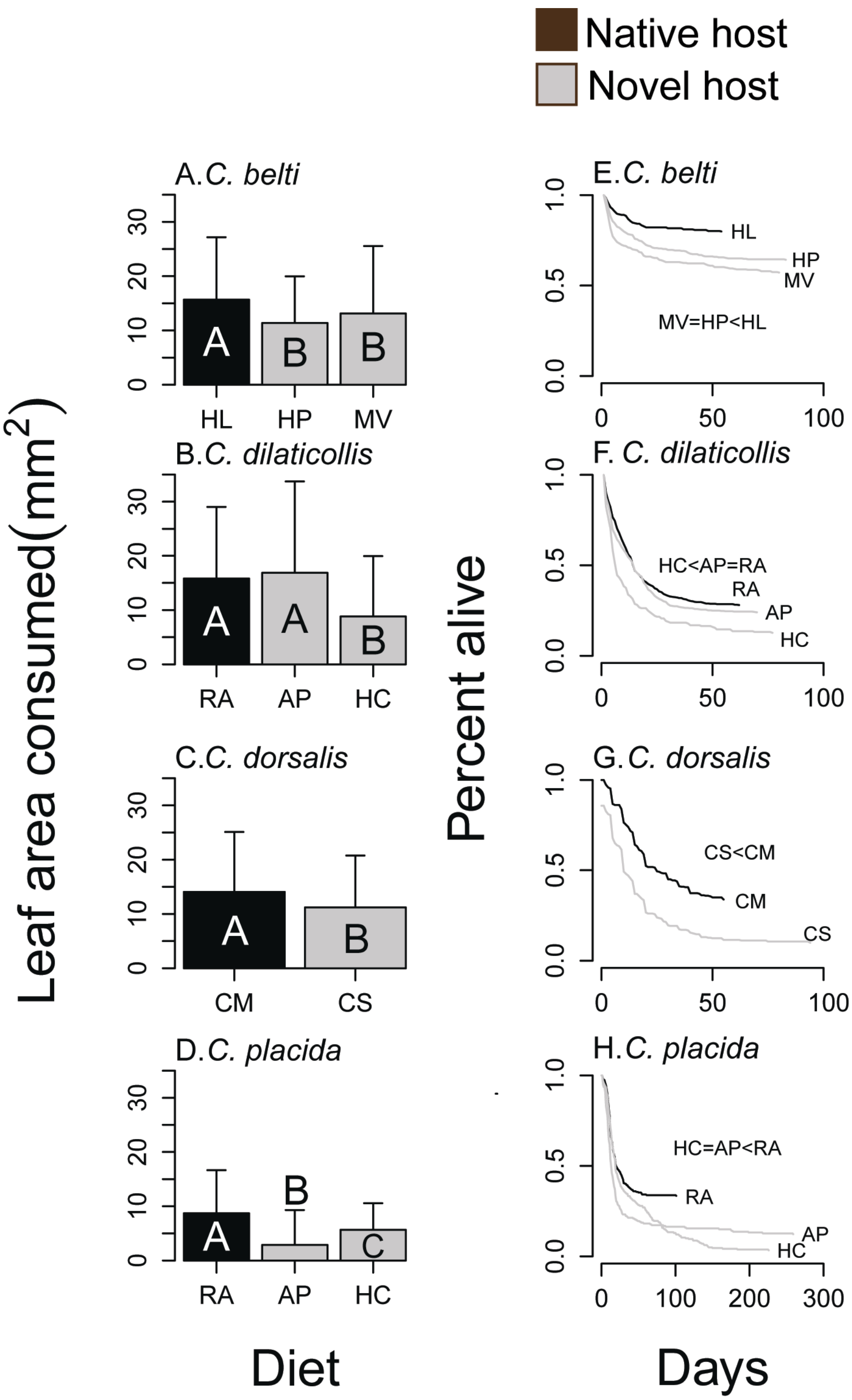
Native host
Novel host
A.C. belti
B.C. dilaticollis
C.C. dorsalis
D.C. placida
Leaf area consumed(mm^2)
0
10
20
30
HL
HP
MV
RA
AP
HC
CM
CS
A
B
C
Diet
E.C. belti
HL
HP
MV
MV=HP<HL
F. C. dilaticollis
HC<AP=RA
RA
AP
HC
G.C. dorsalis
CS<CM
CM
CS
H.C. placida
HC=AP<RA
RA
AP
HC
Percent alive
0.0
0.5
1.0
0
50
100
200
300
Days

Figure 5.1. Larval acceptability (Mean ± SD) and survival of *Cephaloleia* beetles on native and novel host plants. A. and E. Acceptability and survival of the generalist beetle *C. belti* on the native host *H. latispatha* (HL) and the novel hosts *H. psittacorum* (HP) and *M. velutina* (MV). B. and F. Acceptability and survival of the generalist beetle *C. dilaticollis* on the native host *R. alpinia* (RA) and the novel hosts *A. purpurata* (AP) and *H. coronarium* (HC). C and G. Acceptability and survival of the specialist beetle *C. dorsalis* on the native host *C. malortieanus* (CM) and the novel host *Ch. speciosus* (CS). D. and H. Acceptability and survival of the specialist beetle *C. placida* on the native host *R. alpinia* (RA) and the novel hosts *A. purpurata* (AP) and *H. coronarium* (HC).

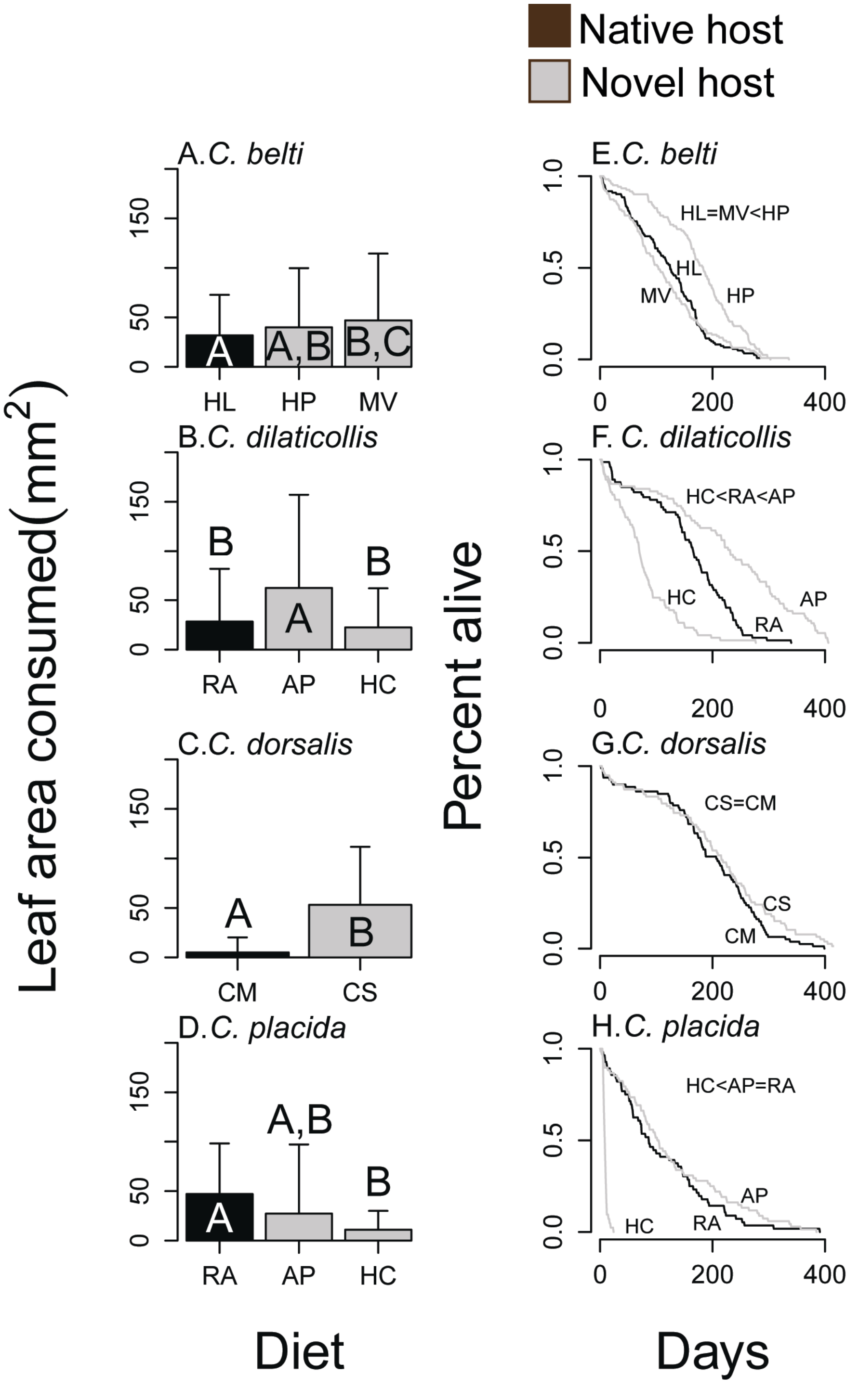
Native host
Novel host
Leaf area consumed(mm^2)
A. C. belti
150
50
0
A
A,B
B,C
HL
HP
MV
B. C. dilaticollis
B
A
B
RA
AP
HC
C. C. dorsalis
A
B
CM
CS
D. C. placida
A
A,B
B
RA
AP
HC
Diet
Percent alive
E. C. belti
1.0
0.5
0.0
HL=MV<HP
HL
MV
HP
0
200
400
F. C. dilaticollis
HC<RA<AP
HC
RA
AP
G. C. dorsalis
CS=CM
CS
CM
H. C. placida
HC<AP=RA
AP
HC
RA
Days

Figure 5.2. Adult preference (Mean ± SD) and survival of *Cephaloleia* beetles in native and novel host plants. A. and E. Preference and survival of the generalist beetle *C. belti* in the native host *H. latispatha* (HL) and the novel hosts *H. psittacorum* (HP) and *M. velutina* (MV). B. and F. Preference and survival of the generalist beetle *C. dilaticollis* in the native host *R. alpinia* (RA) and the novel hosts *A. purpurata* (AP) and *H. coronarium* (HC). C and G. Preference and survival of the specialist beetle *C. dorsalis* in the native host *C. malortieanus* (CM) and the novel host *Ch. speciosus* (CS). D. and H. Preference and survival of the specialist beetle *C. placida* in the native host *R. alpinia* (RA) and the novel hosts *A. purpurata* (AP) and *H. coronarium* (HC).

Chapter VI

Experimental demography of host colonization by generalist and specialist herbivores: The plasticity of life-history traits in novel environments[5]

SUMMARY

Colonization success of species when confronted with totally novel environments is of interest in ecological, evolutionary and conservation contexts. Such events may represent the first step for ecological diversification and, when accompanied by differential selection and genetic responses, they may play an important role in adaptive divergence and speciation. The breadth of environments in which a species can succeed is ultimately determined by the full pattern of its vital rates in each environment. A species that is able to do well across a range of environments is said to be more plastic (*sensu lato*) than one whose success is restricted to a single or to very few environments. The breadth of environments in which a species can succeed is ultimately determined by the full pattern of its vital rates in each environment. Examples of organisms colonizing novel environments are insect herbivores expanding their diets to novel host plants. One expectation for insect herbivores is that species with specialized diets may exhibit less plasticity when faced with novel hosts than generalist species. We examine this hypothesis for two generalist and two specialist neotropical beetles (genus *Cephaloleia*: Chrysomelidae) currently expanding their diets from native to exotic plants from the order Zingiberales in a Central American tropical rain forest (La Selva Biological Station, Costa Rica). Using an experimental approach, we estimated changes in life history traits, vital rates and lifetime fitness, measured by *r* (the instantatatenous population growth rate) for each beetle species in native and novel host plants. We did not find evidence

supporting more plasticity for generalist than for specialist species. Larvae survived worse on most novel hosts; adults survived at least as well or better, but reproduced less on the novel host than on natives. Population growth was reduced on all the novel hosts. Some of the novel host plants represent particularly challenging habitats where population growth was negative. However, in four of the novel plant-herbivore interactions included in this study, instantaneous population growth rates were positive. This suggests that the plasticity of life history traits allows both generalist and specialist herbivores to successfully colonize novel hosts without apparent evolutionary changes prior to diet expansion.

BACKGROUND

Many species face heterogeneous environments within the lifetimes of individuals as well as among cohorts and across populations (Thomas et al. 1987, Hawthorne 1997, Law and Dickman 1998, Sax and Brown 2000, Yeh and Price 2004, Thomas et al. 2009, Verhoeven et al. 2009). Individuals may experience such heterogeneity at different temporal and spatial scales, ranging from daily movements among adjacent habitats to seasonal use of geographically separated environments (Gardner et al. 2009). Environmental heterogeneity may arise from differences in abiotic or biotic factors among localities or resource patches (Caswell 1983, Hawthorne 1997). A species that is able to do well across a range of environments is said to be more plastic (*sensu lato*)

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than one whose success is restricted to a single or to very few environments. The breadth of environments in which a species can succeed is ultimately determined by the full pattern of its vital rates in each environment, but this is rarely known (Caswell 1983).

In the widest sense, phenotypic plasticity refers to the ability of an organism to change its phenotype in response to changes in the environment. Often, researchers are interested in particular morphological forms or changes within the lifetime of an individual, but here we are interested in the life history traits themselves and differences that can occur among cohorts that experience different environments, including the statistics derived from cohort life tables: age at first reproduction, net reproductive rate, generation time, life expectancy from birth, life expectancy after reaching reproductive age, and intrinsic instantaneous population growth rate. One expectation for insect herbivores is that species observed to feed on few host plants in nature would exhibit less plasticity when faced with novel hosts than species observed to feed on many host plants in nature (Powell 1971, Powell and Wistrand 1978, Hawthorne 1997). In this paper, we examine this hypothesis using an experimental approach, obtaining data on the full life cycle of four beetles (two specialist and two generalist) in native and novel environments.

Colonization success of species when confronted with totally novel environments is of interest in ecological, evolutionary and conservation contexts (Keane and Crawley 2002). Such events may represent the first step for ecological diversification and, when accompanied by differential selection and genetic responses, they may play an important role in adaptive divergence and speciation (Futuyma and Moreno 1988, Orr and Smith 1998). The conservation status of species may be directly affected when original environments are altered due to fragmentation, biological invasion and climate-changeinduced geographic shifts. Under these alterations, organisms may have to colonize novel environments if they are to persist at all (Willis and Bhagwat 2009). Estimating the full set of vital rates (age-specific birth rates and death rates over the entire life cycle) of populations colonizing novel environments is challenging (Reznick and Ghalambor 2001).

The challenge by any organism during early colonization is the ability to persist and/or flourish in the novel environment (Sax and Brown 2000, Keane and Crawley 2002, Verhoeven *et al.* 2009). Population growth rate during early colonization is of particular interest (Birch 1948). Population growth rate is a parameter that links ecology and evolution, integrating the effects of multiple factors affecting the organism across its life cycle (Mayhew 2001, Fordyce 2006). Negative population growth rate indicates eventual extinction in the novel environment (Birch 1948, Caswell 1983). Non-negative population growth rates indicate persistence or growth and therefore an opportunity for future adaptation to the novel environment (Birch 1948, Caswell 1983).

In neotropical rain forests, *Cephaloleia* beetles (Chrysomelidae, Cassidinae) are herbivores of plants in the Zingiberales (Staines 1996). This is one of the oldest and most conservative plant-herbivore interactions (Wilf et al. 2000). *Cephaloleia* beetles and Zingiberales diversified in the Neotropics over the last 40 – 60 MY (Wilf *et al.* 2000, Gómez-Zurita et al. 2007, García-Robledo and Staines 2008).

During the last decade, five exotic Zingiberales from the Paleotropics and South America were introduced to La Selva Biological Station, a tropical rain forest in Costa Rica (Central America). At least 7 species of *Cephaloleia* are currently in the process of expanding their diets to these exotic host plants, generating 16 novel plant-herbivore interactions (García-Robledo et al. in press). These incipient diet expansions present an opportunity to study the response of life history traits to novel environments, in this case exotic host plants. The *Cephaloleia* which have been observed on these novel hosts span the range of diet breadths observed in the genus, ranging from those who are specialized on a few species in a single plant family to those who feed on as many as 15 plant species across three families (García-Robledo *et al.* In press). The contrasting diet breadths present the opportunity to test the prediction that generalist species will be more plastic when colonizing novel environments than specialists. By this we mean that on novel hosts, we expect generalists to perform as well as they would on historical hosts, while we expect specialists to perform less well on novel hosts than they do on historical hosts.

In this research, we selected two generalist and two specialist *Cephaloleia* beetles, all of whom have been observed to make use of the novel, exotic host plants introduced into their native habitat (Table 6.1). To understand the role of environmental-driven changes of life history traits on the demography of generalist and specialist herbivores colonizing novel environments, the objectives of this research include the following. For each beetle species, we determined the degree of divergence between life history traits measured on beetles raised on historical hosts compared to life history traits for beetles raised on novel host plants, using an experimental demography approach.

METHODS

STUDY SITE AND SPECIES — This study was conducted from August 2005 to March 2009 at La Selva Biological Station (hereafter La Selva), a tropical rain forest in Costa Rica, Central America (10º26’N, 83º59’W). We selected four *Cephaloleia* beetle species with contrasting diet breadths as study species (Table 6.1). At La Selva, *Cephaloleia belti* is the species with the broadest diet breadth, feeding on 15 species from three families of Zingiberales (García-Robledo et al. In press). The beetle *Cephaloleia dilaticollis* is also a generalist, feeding on ten species from three families of Zingiberales (García-Robledo et al. In press).

We also selected two species with specialized diets. *Cephaloleia dorsalis,* that feeds on at least four species in the family Costaceae and *Cephaloleia placida* feeds on at least two species in the family Zingiberaceae (García-Robledo *et al.* in press).

Adults of the four *Cephaloleia* species feed on the leaf tissue of the young rolled leaves of their host plants. In contrast, larvae feed on the leaf tissue of expanded leaves (García-Robledo *et al.* in press). The four beetle species are currently expanding their diets to exotic hosts from India, the Malay Peninsula, the Pacific Islands and South America (Table 6.1).

SURVIVAL OF IMMATURE STAGES IN NATIVE AND EXOTIC HOST PLANTS — To determine if survival of *Cephaloleia* larvae differ between native or novel host plants, we performed the following experiment. We collected males and females of each *Cephaloleia* species from the native host plants where larvae and adults of each beetle species are most frequently associated (García-Robledo *et al.* in press; Table 6.1). Mating couples were placed in separate 17 X 15 X 5 cm containers and fed *ad libitum* with leaf tissue from their native host plants (Number of females: $N_{C.belti} = 38$, $N_{C.dilaticollis} = 32$, $N_{C.dorsalis} = 37$, $N_{C.placida} = 42$)**.** Eggs were collected and after eclosion, newborn larvae were randomly assigned to one of the following diets: leaf tissue from the native host or leaf tissue from the novel host plant (Table 6.1). Each larva was placed in an individual container and fed every 48 h with two 3.5 cm diameter disks of leaf tissue. Larvae were reared at a mean temperature of 27°C and a light regime of 12 h. light 12 h. darkness. (Sample size *C. belti*: $N_{\textit{H. latispatha}} = 334$, $N_{\textit{H. psittacorum}} = 374$, $N_{\textit{M. velutina}} = 391$; *C. dilaticollis*: $N_{\textit{R. alpinia}} = 545$, $N_{\textit{A. purpurata}} = 526$, $N_{\textit{H. coronarium}} = 184$; *C. dorsalis*; $N_{\textit{C. malortieanus}} = 337$, $N_{\textit{Ch. speciosus}} = 316$; *C. placida*: $N_{\textit{R. alpinia}} = 268$, $N_{\textit{A. purpurata}} = 214$, $N_{\textit{H. coronarium}} = 239$).

To estimate larval survival in native and novel host plants, we monitored each larva every 48 h until death or pupation. Differences in larval mortality between native and novel hosts were determined by Kaplan-Meyer survival analyses.

EFFECT OF DIET ON LARVAL AND PUPAL DEVELOPMENT TIME — To determine larval development times in native and novel host, we performed the following experiments. For each larva selected for the survival experiments, we recorded time to pupation (*C. belti*: $N_{\textit{H. latispatha}} = 278$, $N_{\textit{H. psittacorum}} = 250$, $N_{\textit{M. velutina}} = 228$; *C. dilaticollis*: $N_{\textit{R. alpinia}} = 143$, $N_{\textit{A. purpurata}} = 130$, $N_{\textit{H. coronarium}} = 27$; *C. dorsalis:* $N_{\textit{C. malortieanus}} = 115$, $N_{\textit{Ch. speciosus}} = 80$; *C. placida*: $N_{\textit{R. alpinia}} = 91$, $N_{\textit{A. purpurata}} = 26$, $N_{\textit{H. coronarium}} = 9$). We also recorded times from pupation to adult eclosion in larvae reared in the native and novel hosts (*C. belti*: $N_{\textit{H. latispatha}} = 210$, $N_{\textit{H. psittacorum}} = 173$, $N_{\textit{M. velutina}} = 163$; *C. dilaticollis*: $N_{\textit{R. alpinia}} = 74$, $N_{\textit{A. purpurata}} = 75$, $N_{\textit{H. coronarium}} = 11$; *C. dorsalis:* $N_{\textit{C. malortieanus}} = 84$, $N_{\textit{Ch. speciosus}} = 58$; *C. placida*: $N_{\textit{R. alpinia}} = 71$, $N_{\textit{A. purpurata}} = 14$). Differences in development times among treatments were explored using one-way ANOVA's or Welch two-samples t-tests.

EFFECTS OF LARVAL DIET ON ROLLED-LEAF BEETLE SEX RATIO — To determine if the sex ratio of adults obtained from larvae reared in the native or novel hosts depart from a 1:1 proportion, we recorded the gender of each adult reared in the laboratory. Gender identification is possible for the four *Cephaloleia* species as they display sexual dimorphism in the last abdominal sternite (Garcia-Robledo et al. in press). (*C. belti*: $N_{\mathit{H.\ latispatha}} = 207$, $N_{\mathit{H.\ psittacorum}} = 169$, $N_{\mathit{M.\ velutina}} = 158$; *C. dilaticollis*: $N_{\mathit{R.\ alpinia}} = 74$, $N_{\mathit{A.\ purpurata}} = 72$, $N_{\mathit{H.\ coronarium}} = 7$; *C. dorsalis:* $N_{\mathit{C.\ malortieanus}} = 82$, $N_{\mathit{Ch.\ speciosus}} = 44$; *C. placida*: $N_{\mathit{R.\ alpinia}} = 69$, $N_{\mathit{A.\ purpurata}} = 14$). Differences in sex ratios in larvae reared in native and novel hosts were tested using chi-square analyses.

EFFECTS OF DIET AS A LARVA, DIET AS ADULT AND GENDER ON ADULT LONGEVITY — To determine the effects of diet as a larva, diet as adult and gender on the longevity of *Cephaloleia* beetles, we performed the following experiment. We obtained adults of each beetle species from larvae reared in native or novel hosts (Table 6.1). The gender of each adult was recorded before placed in individual containers. Adults were fed *ad libitum* with leaf tissue of either their native or their novel host plant (Table 6.1). Time to death was recorded by monitoring each beetle every 48 h for 429 days. We explored differences in adult survival among diets with a fully crossed ANOVA design that included diet as larva, diet as adult and gender as fixed factors. The response variable was the square root-transformed time to death.

EFFECT OF DIET ON FEMALE FECUNDITY — To determine the effect of diet on female fecundity, we obtained females of the four *Cephaloleia* species from larvae reared in either their native or novel hosts (*C. belti*: $N_{H.\ latispatha} = 27$, $N_{H.\ psittacorum} = 31$, $N_{M.\ velutina} = 23$. *C. dorsalis*: $N_{C.\ malortieanus} = 23$, $N_{Ch.\ speciosus} = 20$). Mortalities of larvae and adults of *C. dilaticollis* and *C. placida* reared in the novel host *H. coronarium* were very high (*see* results). For this reason we were able to record the fecundities of *C. dilaticollis* only in the native host *R. alpinia* and the novel host *A. purpurata* (*C. dilaticollis*: $N_{R.\ alpinia} = 6$, $N_{A.\ purpurata} = 5$). We were not able to obtain enough females of *C. placida* from larvae reared in the exotic host *A. purpurata* because larval mortality was very high (*see* results). For this reason estimates of female fecundity in the novel host plant *A. purpurata* were obtained from females reared as larva in the native host *R. alpinia* (*C. placida*: $N_{R.\ alpinia} = 20$, $N_{A.\ purpurata\ \text{(from larvae reared in}\ R.\ alpinia\text{)}} = 14$).

Females were placed in individual containers with two males. Females were fed *ad libitum* every 48 h with young leaf tissue from the host plant where individuals were reared during larval development. In addition, four 10 x 10 squares of old leaves were included in the box as oviposition substrate. The number of eggs laid and larvae produced by each female were recorded weekly for seven weeks.

To determine differences in fecundity between females reared in native or novel hosts, we performed a Mixed-effects Repeated Measures ANOVA. The model allows within group errors with unequal variances (R-Development-Core-Team 2009). The model including diet (native vs novel host plants) as a fixed factor, and the number of larvae produced each week for each female as a repeated measure. A posteriori comparisons were performed using the General linear Hypothesis test (Package multcomp, R-Development-Core-Team 2009).

Cohort life table statistics for historical and novel environments — For each beetle species-host plant combination, we estimated the following vital rates from cohort life tables constructed from experiments: generation time (T), age at first reproduction (a), net reproductive rate (R_0), life expectancy (e_0) for newborn larvae, life expectancy for those that survived reproductive age, (e_a) and intrinsic instantaneous population growth rate (r). Vital rates in different host plants were estimated by combining the results of the following experiments.

For the four *Cephaloleia* species (Table 6.1) we obtained in the laboratory larvae that were placed in individual containers and assigned to one of the following diets: leaf tissue from the native host or from the novel host plants (Table 6.1). In this experiment, all individuals assigned to the same diet represent a cohort (*see* sample size in Figure 6.5, Table 6.4). We fed individuals of each cohort every 48 h with two 3.5 cm d disks of leaf tissue from expanded leaves. For each larva we recorded the time to death or to pupation. Individuals that pupated were placed in individual containers. We weekly recorded the pupae that died. For the individuals that survived we recorded the time from pupation to adult eclosion.

Adults were placed in individual containers and fed *ad libitum* every 48 h. with young leaf tissue from the same host plants that was assigned during the larval stage. We recorded for individuals within each cohort the time to death.

For each cohort of individuals reared in the native or the novel hosts we generated an event history diagram (Carey 2003; Figure 6.5). These diagrams represent the empirical survival functions for each species in the different environments (Carey 2003)

Because there is no difference in longevity between genders, for the generalist species *C. belti* and *C. dilaticollis,* (*see* results) we included event histories of both males and females in the estimation of the empirical survival functions. Males of the specialist species *C. dorsalis* and *C. placida* live longer than females (García-Robledo *et al.* in press). Therefore for these species only female event histories were included in the estimates of the empirical survival functions.

Using the empirical survival functions, we estimated l_x, the proportion of individuals alive at week x for cohorts reared in native or exotic host plants (Birch 1948). Using the fecundity estimates (see results, Figure 6.4) and the sex ratios of individuals reared in native or novel hosts (see results, Figure 6.3), we calculated m_x, the mean number of daughters born by each female in week x (Birch 1948). Note that we only measured female fecundities during their first seven weeks of life. For this reason, we generated two estimates for the parameters affected by changes in m_x.: a. The minimum potential value, estimated assuming that females don't reproduce after the seventh week of life and b. The maximum potential value for each parameter, that assumes equivalent fecundity from the seventh week of life until females' death.

For each *Cephaloleia* species we recorded the age at first reproduction in native and novel host plants. Estimates of age at first reproduction are fundamental to understand changes in generation time in native and novel environments (Carey 1993, 2003). Combining the survival and fecundity estimates, we calculated for each cohort T, the generation time (Equation 1), *i.e.* the mean length of generations in native or novel hosts (Birch 1948):

$$T = \frac{\sum x l_x m_x}{\sum l_x m_x} \qquad (1)$$

We also estimated R_0, the net reproduction rate (Equation 2), This represents the mean offspring per female produced in each environment (Birch 1948).

$$R_0 = \sum l_x m_x \qquad (2)$$

We estimated the mean life expectancy (e_0) from a. larval eclosion, and b. from the age of first reproduction (e_a) (Equation 3, Carey 1993)). T_x represent the expected number of days lived beyond week x. L_x is the proportion of the native cohort still alive during the age interval x to x+1 weighted by the length of the interval. T_x is the sum of these L_x's, from time x to the time of death of all individuals in the cohort (Equation 4, Carey 1993).

$$e_x = \mathrm{T}_x / l_x \qquad (3) \qquad\qquad \mathrm{T}_x = \sum_{y=x}^{\varpi} L_y \qquad (4)$$

Finally, we estimated for each cohort reared in native and novel hosts the instantaneous population growth rate r (Equation5, Birch 1948).

$$r = \frac{\ln R_0}{T} \qquad (5)$$

RESULTS

SURVIVAL OF IMMATURE STAGES IN NATIVE AND NOVEL HOST PLANTS — Larval survival of *C. belti* is higher in the original host *H. latispatha* than in the novel hosts *H. psittacorum* and *M. velutina* ($\chi^2 = 44.82$, DF = 2, $P < 0.001$ Figure 6.1A). Pupal survival is equivalent among host plants ($\chi^2 = 2.6742$, df = 2, $P = 0.26$, Figure 6.1B)

Larval survival of *C. dilaticollis* is equivalent in the original host *R. alpinia* and in the novel hosts *A. purpurata*. Larval suvival is lower in the novel host *H. coronarium* ($\chi^2 = 26.02$, DF = 2 , $P < 0.001$ Figure 6.1D). Pupal survival is equivalent among hosts ($\chi^2 = 3.0677$, df = 2, $P = 0.21$Figure 6.1E).

Larval survival of *C. dorsalis* is higher in the original host *C. malortieanus* than in the novel host *Ch. speciosus* ($Z = 3.44$, DF = 1, $P < 0.001$ Figure 6.1G). Pupal survival is equivalent between hosts ($\chi^2 = 0.016$, df = 1, $P = 0.90$, Figure 6.1H).

Larval survival of *C. placida* is higher in the original host *R. alpinia* than in the novel hosts *A. purpurata* and *H. coronarium* ($\chi^2 = 28.78$, DF = 2 , $P < 0.001$ Figure 6.1J). For this species pupal survival is higher in the original host plant ($\chi^2 = 15.474$, df = 2, $P < 0.001$, Figure 6.1K).

EFFECT OF DIET ON LARVAL AND PUPAL DEVELOPMENT TIME — Larval development time of *C. belti* is shorter in the original host *H. latispatha* than in the exotic hosts *H. psittacorum* and *M. velutina* ($F_2 = 115.17$, $P < 0.0001$, Figure 6.2A). Time as a pupa is equivalent among hosts ($F_2 = 1.99$, $P < 0.14$, Figure 6.2B).

In *C. dilaticollis,* larval development time is shorter in the original host *R. alpinia* than in the exotic hosts *A. purpurata* and *H. coronarium* ($F_2 = 26.35$, $P < 0.0001$, Figure 6.2C). Time as a pupa is equivalent among hosts ($F_2 = 0.26$, $P < 0.77$, Figure 6.2D).

Larval development time of *C. dorsalis* is shorter in the original host *C. malortieeanus* than in the novel host *Ch. speciosus* ($t_{153.88} = -4.26$, $P < 0.0001$, Figure 6.2E). Time as a pupa is equivalent among hosts ($t_{124.9} = 0.22$, $P < 0.82$, Figure 6.2F).

In *C. placida,* larval development time is shorter in the original host *R.* alpinia than in the exotic hosts *A. purpurata* an *H. coronarium* ($F_2 = 72.92$, $P < 0.0001$, Figure 6.2G). Time as a pupa is equivalent among hosts ($t_{34.83} = 0.10$, $P < 0.92$, Figure 6.2H).

EFFECTS OF LARVAL DIET ON *CEPHALOLEIA* BEETLES SEX RATIO — The sex ratios of individuals of *C. belti* reared in the original host *H. latispatha* and the exotic hosts *H. psittacorum* and *M. velutina* do not deviate from 1:1 ratios (*H. latispatha*: $\chi^2 = 0.237$, df = 1, P = 0.63; *H. psittacorum*: $\chi^2 = 0.148$, df = 1, P = 0.70; *M. velutina*: $\chi^2 = 0.4051$, df = 1, P = 0.52, Figure 6.3A). The sex ratio of *C. dilaticollis* reared in the original host *R. alpinia* and the exotic hosts *A. purpurata* and *H. coronarium* do not deviate from 1:1 ratios (*R. alpinia*: $\chi^2 = 0.054$, df = 1, P = 0.82; *A. purpurata*: $\chi^2 = 0.5$, df = 1, P = 0.48; *H. coronarium*: $\chi^2 = 0.143$, df = 1, P = 0.70, Figure 6.3B).

The sex ratio of individuals of *C. dorsalis* reared in the original host *C. malortieeanus* does not deviate from a 1:1 ratio ($\chi^2 = 0.439$, df = 1, P = 0.51, Figure 6.3C). However, for the individuals of *C. dorsalis* reared in the exotic host *Ch. speciosus*, we observed a highly skewed sex ratio towards males ($\chi^2 = 7.36$, df = 1, P = 0.007, Figure 6.3C). The sex ratio of *C. placida* reared in the original host *R. alpinia* and the exotic host *A. purpurata* do not deviate from 1:1 ratios (*R. alpinia*: $\chi^2 = 0.71$, df = 1, P = 0.40; *A. purpurata*: $\chi^2 = 0.00$, df = 1, P = 1, Figure 6.3D). We did not test for differences in sex ration in adult *C. placida* reared in the novel host *H. coronarium,* as we only obtained two adults reared in this host.

EFFECTS OF DIET AS LARVA, DIET AS ADULT AND GENDER ON ADULT LONGEVITY — Larval diet does not affect adult longevity in *C. belti, C. dilaticollis* or *C. dorsalis* (Table 6.2). Larvae of *C. placida* reared in exotic hosts displayed a very low survival (Figure 6.1 J). Therefore, we were not able to test the effect of diet as larvae on adult survival for *C. placida*.

Adult *C. belti*, *C. dilaticollis* and *C. placida* displayed differences in longevity when feeding on native or exotic hosts (Table 6.2). In general, adult longevity was higher in the exotic hosts (Figure 6.1 C, F, L). Longevity of adults of *C. dorsalis* feeding on *C. malortieanus* and *Ch. speciosus,* and adults of *C. placida* feeding on *R. alpinia* and *A. purpurata* are equivalent (Figure 6.1 I, L; Table 6.2). Adult mortality of *C. dilaticollis* and *C. placida* in the exotic host plant *H. coronarium* is high (Figure 6.1 F, L).

There are no differences in survival between males and females of the generalist species *C. belti* and *C. dilaticollis* (Table 6.2). However, males of the specialist species *C. dorsalis* and *C. placida* live longer than females (Mean ± SD: $♀_{C.\ dorsalis} = 162.0 \pm 83.7$ d, $♂_{C.\ dorsalis} = 223.58 \pm 97.7$ d; $♀_{C.\ placida} = 78.8.0 \pm 64.3$ d, $♂_{C.\ placida} = 98.8 \pm 114.3$ d, Table 6.2).

Most of the interactions among factors are not significant (Table 6.2). We only found significant interactions between diet as adult and gender for *C. dilaticollis*. In the original host *R. alpinia* and the exotic host *H. coronarium,* adult males live longer than females (*R. alpinia*: ♀ = 151.6 d, ♂ = 164.7 d; *H. coronarium* ♀ = 73.0 d, ♂ = 81.5 d). However, in the novel host *A. purpurata* females live longer than males (♀ = 219.3.0 d, ♂ = 173.7 d).

EFFECT OF DIET ON FEMALE FECUNDITY AND AGE AT FIRST REPRODUCTION — Age at first reproduction in *C. belti* increased in the novel hosts (Table 6. 4). Females also display different fecundities when feeding on different host plants (Table 6.3). Females display the highest fecundity in the original host *H. latispatha,* lower fecundity in the novel host *H. psittacorum* and even lower fecundity in the novel host *M. velutina* (Multiple comparisons test: HL – HP, Z = -2.23, P = 0.07; HL – MV = Z = -4.07, P < 0.001; HP –MV = Z = -2.02, P = 0.11, Host plant abbreviation as in Figure 6.1). *Cephaloleia belti* fecundity increases with female age, reaching a maximum in the sixth week after adult eclosion. Fecundity decreases in subsequent weeks (Figure 6.4A).

Age at first reproduction in *C. dilaticollis* is higher in the novel host *A. purpurata* (Table 6.4). Female *C. dilaticollis* display equivalent fecundities in the original host *R. alpinia* and the novel host *A. purpurata* (Table 6.3). We did not record fecundities in the novel host *H. coronarium* because larval mortality in this host is very high (Figure 6.1D). Fecundities of *C. dilaticollis* females increase during the first five weeks of life, and remain constant in weeks 6 and 7 (Figure 6.4B).

Age at first reproduction in *C. dorsalis* is equivalent for females reared in the original and the novel hosts (Table 6.4). Female *C. dorsalis* display equivalent fecundities in the original and novel hosts (Table 6.3). We observed an increase in the fecundities of females until they were three weeks old. Fecundity is equivalent in females that are four to seven weeks old (Figure 6.4C).

Age at first reproduction in the novel host *A. purpurata* is nine weeks later than in the original host *R. alpinia* (Table 6.4). Fecundities of female *C. placida* were higher in the original than in the novel host (Table 6.4; Figure 6.4C). Fecundities increased as females aged, reaching a maximum when females were five weeks old (Figure 6.4c). In subsequent weeks females displayed a reduction in their fecundities. There is a significant interaction between adult diet and female age (Table 6.3).

DEMOGRAPHIC EFFECTS OF DIET EXPANSIONS TO NOVEL HOSTS IN GENERALIST AND SPECIALIST HERBIVORES — Individuals from the cohort of *C. belti* reared in the original host *H. latispatha* display shorter larval development times than individuals reared in the novel hosts (Figure 6.5 A – C). The empirical survival functions show higher survival in the original host than in the exotic hosts (Figure 6.5 A – C). The life table analyses combining the empirical survival functions (Figure 6.5 A – C) and the fecundity estimates (Figure 6.4A) show that the shortest generation time corresponds to beetles reared in the original host *H. latispatha* and the novel *M. velutina* (Table 6.4). Generation times in the original host *H. latispatha* and the novel host *M. velutina* are 1.5 – 3 weeks shorter than in the novel host *H. psittacorum* (Table 6.4). Individuals of *C. belti* reared in the original host *H. latispatha* display the highest net reproduction rate (Table 6.4).

The life expectancy for newborn larvae of *C. belti* is higher in the original than in the novel hosts (Table 6.4). In the novel host, life expectancy decreases for adults in reproductive age. However, life expectancy increases for reproductive adults in the novel host plants (Table 6.4). In this species, all the estimated instantaneous population growth rates are positive (Table 6.4). The instantaneous population growth rate is higher for beetles feeding on the original host than for beetles feeding on the novel hosts (Table 6.4).

Individuals of *C. dilaticollis* reared in the original host *R. alpinia* displayed shorter larval development times than individuals reared in novel hosts (Figure 6.5 D - F). Survival of individuals reared in the original host *R. alpinia* and the exotic host *A. purpurata* is higher than the survival of individuals reared in the exotic host *H. coronarium* (Figure 6.5 D - F). Generation time in the original host is five weeks shorter than in the exotic host *A. purpurata* (Table 6.4). The net reproduction rate in the original host *Renealmia alpinia* is highly reduced when assuming that females don't reproduce after the seventh week of life.

The life expectancy for newborn larvae of *C. dilaticollis* is higher in the original than in the novel host (Table 6.4). Life expectancy increases for adults in reproductive age in both the original and the novel host plants (Table 6.4). The instantaneous population growth rate is lower in the cohort reared in *R. alpinia* assuming no reproduction higher in the cohort reared in the original host *R. alpinia* than in the cohort reared in *A. purpurata* (Table 6.4). Survival of *C. dilaticollis* in the novel host *H. coronarium* is very low (Figure 6.4F). In this host the net reproduction rate is zero (Table 6.4).

Individuals of *C. dorsalis* reared in the original host *C. malortieanus* displayed shorter larval development times than individuals reared in novel host *Ch. speciosus* (Figure 6.5 G - H). Survival of individuals reared in the original host is higher than the survival of individuals reared in the exotic host (Figure 6.5 G - H). Generation times of individuals reared in the original and novel hosts are similar (Table 6.4). The net reproduction rate of individuals reared in the original host is almost two times higher than for individuals reared in the novel host (Table 6.4).

The life expectancy for newborn larvae is equivalent in the original and the novel hosts (Table 6.4). Life expectancy increases for adults in reproductive age in both the original and the novel host plants (Table 6.4). The instantaneous population growth rate is higher in the cohort reared in the original host *C. malortieanus* than in the cohort reared in *Ch. speciosus* (Table 6.4).

Individuals of *C. placida* reared in the original host *R. alpinia* displayed shorter larval development times than individuals reared in novel hosts (Figure 6.5 I - K). Survival of individuals reared in the original host *R. alpinia* and the exotic host *A. purpurata* is higher than the survival of individuals reared in the exotic host *H. coronarium* (Figure 6.5 I - K). Generation time in the original host is ca. five weeks longer than in the exotic host *A. purpurata* (Table 6.4). The net reproduction rate is higher for cohorts reared in the original host *R. alpinia.*

The life expectancy for newborn larvae of *C. placida* is higher in the original than in the novel host (Table 6.4). Life expectancy increases for adults that reach their reproductive age in both the original and the novel host plants (Table 6.4). The instantaneous population growth rate is positive in the cohort reared in the original host

R. alpinia and negative in the cohort reared in *A. purpurata* (Table 6.4). Survival of *C. placida* in the novel host *H. coronarium* is very low (Figure 6.5K). In this host the net reproduction rate is zero (Table 6.4).

DISCUSSION

In this study we did not find evidence supporting higher plasticity of life history traits in generalist species than in specialist species. Theoretical models predict that the probability of successful colonization of novel habitats increases when individuals within a population are constantly exposed to variable environments (Bradshaw 1965, Levin 1968, Scheiner 1993, Ghalambor et al. 2007). This is assumed to be a consequence of an increase of the adaptive plasticity of life history traits (*i.e.* the plasticity maintained by natural selection), coupled with an increase in genetic variation in populations exposed to several environments (Miner et al. 2005, Ghalambor *et al.* 2007). However, empirical evidence is contradictory, in some cases supporting but in other cases opposing the expectations of theoretical models (Hawthorne 1997, Pigliucci 2005).

Over all, the mean phenotypes of life history traits of generalist and specialists display similar responses when raised on novel hosts. In general, mean larval survival is greatly reduced on novel hosts. Larval development is also slower on novel hosts. Longer larval development times in the novel hosts does not favor colonization of novel environments. For example, a slower development will increase the generation time, reducing population growth. In nature slower development increase the probability of predation by insect predators or parasitoids (Clancy and Price 1987, Loader and Damman 1991, Benrey and Denno 1997).

Distinct from the patterns observed for larval stages, mean adult survival is equivalent in the original and novel hosts, or even higher in the novel host plants. When comparing life expectancies of larvae and adults in the original and novel hosts, newborn larvae in novel hosts usually display shorter life expectancies than newborn larvae reared in the original hosts (Table 6.4). Adults life expectancy in the novel hosts is usually equal or higher than in the original hosts (Table 6.4). These results suggest that adult mean phenotypes are more plastic in novel environments than larval phenotypes. This pattern of higher survival of adult than immature stages in novel environments had been described previously for other herbivore expanding their diets to novel hosts (Scheirs et al. 2000).

The increase in adult longevities observed on novel hosts is not associated with an increase in fecundity. In all *Cephaloleia* species female fecundities in novel hosts is equal or lower than in the original hosts. The reduction of fitness generated by a decrease in fecundity in the novel hosts could be compensated by an increase of longevity of reproductive individuals in the novel environment (Carey 1993). This is not the case for the *Cephaloleia* species included in this study. The net reproduction rates, *i.e.* the mean number of descendants per individual during their lifetime are always smaller in the novel than in the original host plants (Table 6.4).

For most *Cephaloleia* species, sex ratio is equivalent in cohorts reared in the original and novel host plants. The specialist on plants from the family Costaceae, *Cephaloleia dorsalis* is an exception. This species displays a sex ratio close to 1:1 in the original host, *Costus speciosus.* However, the cohort reared in the exotic host *Cheilocostus speciosus* displayed a sex ratio highly skewed toward males. Most of the females reared in thes novel host were deformed. Plants from the family *Costaceae* contain as anti-herbivore defense diosgenin, a steroidal sapogenin, precursor of corticoids, sex hormones and anabolic agents. Leaf tissue of the novel host *Cheilocostus speciosus* has a concentration of diosgenin 10 times higher than leaves of the original host *Costus speciosus* (Gupta et al. 1981, Nagendra Prasad and Janaki Ammal 1983). It is possible that this hormone precursor is the cause of the observed differences in mortality between genders, and the deformities of females reared in the novel host (Gupta et al. 1981, Nagendra Prasad and Janaki Ammal 1983).

Generation time, the average time span between the birth of an individual and the birth of its offspring, can either increase or decrease in cohorts of *Cephaloleia* beetles reared on the novel hosts (Table 6.1). Generation time can increase as a consequence of an increase in survival, or a delay in the age at first reproduction (Carey 1993, Gaillard et al. 2005, Jones et al. 2008).

For most of the *Cephaloleia* species, differences in generation time between cohorts reared in original or novel hosts are related to changes in survival in the novel environment. Generation time of the generalist beetle species *C. belti* in the novel host *H. psittacorum* is 2.9 weeks longer than in the original host *H. latispatha*. Life expectancy in this plant species is 5.1 weeks longer than in the original host. Females lay eggs two weeks later than females reared on the novel host. Therefore the increase in generation time in this novel host is the combined result of higher survival of adults in the novel host and a delay in the time to first reproduction.

The generation time of *Cephaloleia belti* is 1.5 weeks shorter in the novel host *M. velutina.* In this novel host, the time to first reproduction is delayed one week. This delay in the age at first reproduction contributed in some extent to an increase in the generation time. However, the mortality rate in this novel host is high, reducing generation time.

The cohort of the generalist *C. dilaticollis* reared in the novel host *A. purpurata* displays a generation time five weeks longer than in the original host *R. alpinia*. This increase in generation time is a product of extended adult longevity in this novel host.

Generation time in the specialist *C. dorsalis* is slightly shorter (*i.e.* 0.6 weeks) in the novel than in the original host . Females of *C. dorsalis* start reproduction at similar ages. Therefore, this reduction in generation time is a product of a slight higher mortality in the novel host. In the specialist *C. placida,* generation time is 5.2 weeks shorter in the novel host *A. purpurata* than in the original host *R. alpinia.* This is a consequence of both high mortality and very low fecundity in this novel host.

The novel host *Hedychium coronarium* represents a very challenging environment for both the generalist *C. dilaticollis* and the specialist *C. placida*. High mortality of both larvae and adults, and a net reproductive rate of zero resulted in no generation replacement for these two species in *H. coronarium*.

In all *Cephaloleia* species the colonization of novel hosts is affecting the expression of several life history traits. Consequently, population growth was reduced in all the novel hosts. Some of the novel host plants represent particularly challenging habitats. For example, the instantaneous population growth rate of the specialist *C. placida* in the novel host *A. purpurata* is negative. Cohorts of the generalist *C. dilaticollis* and the specialist *C. placida* colonizing the novel host *H. coronarium* rapidly died without reproducing.

The extinction of populations colonizing novel environments is common, and most of the attempts of colonization of novel habitats usually fail (Lodge 1993). However, if individuals of a population are able to constantly colonize the novel environments from an environment where population growth is positive, the subpopulation in the novel environments will not become extinct. This scenario represents a source-sink dynamic (Hanski 2003). In the case of *Cephaloleia* beetles colonizing novel plants where populations decline, the novel hosts represent source habitats and the novel host sink habitats. Source-sink dynamics can be reduced by natural selection by the evolution of traits that promote the avoidance of the deleterious habitat, or the evolution of physiological traits that increase individuals survival in the sink habitat (Hanski 2003).

Population decline is not the only outcome of colonization of novel hosts in *Cephaloleia* beetles. In four of the novel plant-herbivore interactions included in this study, instantaneous population growth rates are positive. This suggests that the plasticity of life history traits allows both generalist and specialist herbivores to successfully colonize novel hosts without apparent evolutionary changes prior to diet expansion. These results suggest that successful novel plant-herbivore associations are not always the result of processes of adaptation, which may take several generations. These successful novel plant-herbivore interactions seem to be assembled in a very short time by ecological fitting – the process whereby organisms colonize and persist in novel environments, use novel resources, and/or form novel associations with other species as a result of the suites of traits that they carry at the time they encounter the novel condition (Agosta 2006, Agosta and Klemens 2008).

In conclusion, life history traits of both generalist and specialists display similar responses to novel environments. Diet expansions to novel plants are challenging, especially for immature stages. Although the initial outcome of colonization in some novel hosts is extinction, in other novel plants life-history traits already present in the populations promote the rapid incorporation of novel host plants in to herbivore’s diets. This study is a snapshot of some fundamental demographic properties of four insect populations during early colonization of novel host plant. Future success or failure in the colonization of these novel hosts will depend on the initial demographic rates described in this research, natural selection and the evolutionary responses of life history traits in novel environments.

Table 6.1. *Cephaloleia* beetles and the native and novel host plants selected for experimental demography experiments.

Beetle species	Native host plant	Novel host plants	Origin of novel hosts
Cephaloleia belti (Generalist)			
	Heliconia latispatha (Heliconiaceae)	*Heliconia psittacorum* (Heliconiaceae)	Caribbean, northern South America
		Musa velutina (Musaceae)	India
Cephaloleia dilaticollis (Generalist)			
	Renealmia alpinia (Zingiberaceae)	*Alpinia purpurata* (Zingiberaceae)	Pacific islands
		Hedychium coronarium (Zingiberaceae)	Eastern India
Cephaloleia dorsalis (Specialist)			
	Costus malorteanus (Costaceae)	*Cheilocostus speciosus* (Costaceae)	Malay Peninsula of Southeast Asia
Cephaloleia placida (Specialist)			
	Renealmia alpinia (Zingiberaceae)	*Alpinia purpurata* (Zingiberaceae)	Pacific islands
		Hedychium coronarium (Zingiberaceae)	Eastern India

Table 6.2. Results for the fully-crossed three-way ANOVA design testing the effect of diet as larva (native vs novel hosts) diet as adult (native vs novel hosts) and gender on adult longevity.

Beetle species	Source of variation	df	SS	MS	F	P
C. belti (Generalist)						
	Diet as larva (DL)	2	37.521	18.761	1.556	0.213
	Diet as adult (DA)	2	495.150	247.575	20.534	**<0.001**
	Gender (G)	1	4.255	4.255	0.353	0.553
	DL X DA	4	72.807	18.202	1.510	0.199
	DL X G	2	19.559	9.780	0.811	0.445
	DA X G	2	46.507	23.253	1.929	0.147
	DL X DA X G	4	44.636	11.159	0.926	0.449
C. dilaticollis (Generalist)						
	Diet as larva (DL)	1	5.982	5.982	0.376	0.540
	Diet as adult (DA)	2	912.740	456.370	28.721	**<0.001**
	Gender (G)	1	9.316	9.316	0.586	0.445
	DL X DA	2	24.990	12.495	0.786	0.457
	DL X G	1	42.497	42.497	2.674	0.104
	DA x G	2	134.913	67.456	4.245	**0.016**
	DL X DA X G	2	2.266	1.133	0.071	0.931
C. dorsalis (Specialist)						
	Diet as larva (DL)	1	3.254	3.254	0.179	0.673
	Diet as adult (DA)	1	1.534	1.534	0.085	0.772
	Gender (G)	1	186.572	186.572	10.279	**< 0.001**
	DL X DA	1	2.117	2.117	0.117	0.733
	DL X G	1	31.388	31.388	1.729	0.191
	DA x G	1	2.165	2.165	0.119	0.730
	DL X DA X G	1	25.883	25.883	1.426	0.234
C. placida (Specialist)						
	Diet as adult (DA)	2	1394.503	697.252	57.313	**< 0.001**
	Gender (G)	1	49.326	49.326	4.055	**0.046**
	DA x G	2	65.999	32.999	2.713	0.070

Table 6.3. Results for the repeated measures ANOVA design testing the effect of diet as adult (native vs novel hosts) on female fecundities over time (weeks).

Beetle species	Source of variation	DF (num)	DF (den)	F	P
C. belti (Generalist)					
	Diet	2	78	7.503	**0.001**
	Time (weeks)	6	412	13.771	**< 0.001**
	Diet X Time	12	412	1.617	0.084
C. dilaticollis (Generalist)					
	Diet	1	9	0.084	0.779
	Time (weeks)	6	48	4.578	**0.001**
	Diet X Time	6	48	0.619	0.714
C. dorsalis (Specialist)					
	Diet	1	41	0.565	0.457
	Time (weeks)	6	224	25.047	**< 0.001**
	Diet X Time	6	224	0.702	0.648
C. placida (Specialist)					
	Diet	1	32	8.407	**0.007**
	Time (weeks)	6	149	3.250	**0.005**
	Diet X Time	6	149	2.543	**0.023**

Table 6.4. Vital rates of generalist and specialist rolled-leaf beetles in native to novel host plants.

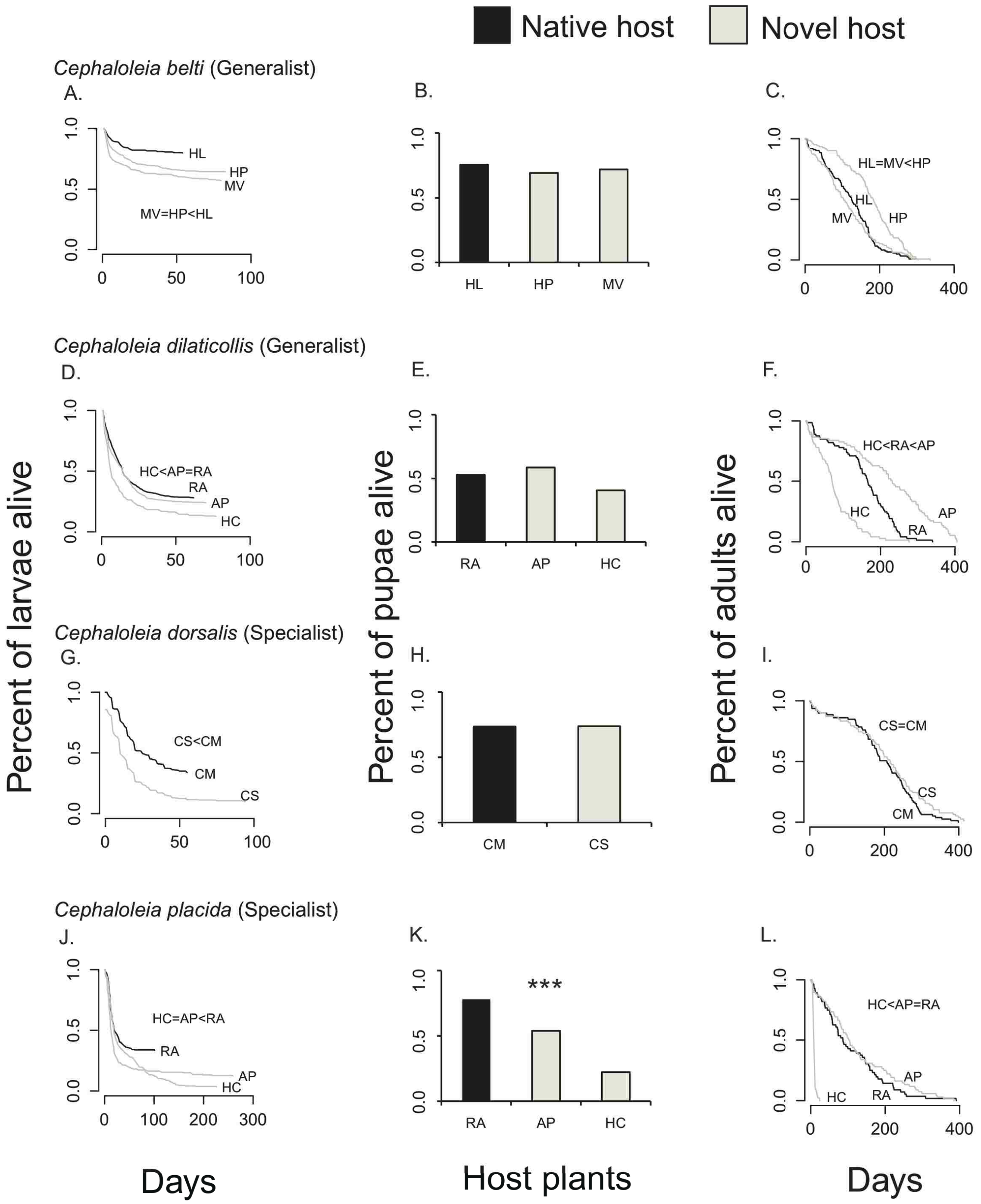
Beetle species	Plant species	N	Generation time (*T*) (Weeks)[a]	Age at first reproduction (Weeks)	Net reproduction rate (R_0)[a]	Life expectancies (e_x) (Weeks) [b]	Instantaneous population growth rate (*r*)[a]
C. belti (Generalist)							
	H. latispatha (Native)	68	12.8 - 20.8	9	4.6 - 14.8	17.1[a], 16.3	0.1189 - 0.1295
	H. psittacorum (Novel)	65	14.3 - 23.7	11	2.4 - 9.5	16.4[a], 21.5	0.0602 - 0.0951
	M. velutina (Novel)	69	12.8 19.3	10	1.23 - 2.7	13.1[a], 15.7	0.0166 - 0.0511
C. dilaticollis (Generalist)							
	R. alpinia (Native)	149	12.23 - 21.9	8	1.49 - 5.5	6.7[a], 21.2	0.0324 - 0.0776
	A. purpurata (Novel)	178	26.9 - 26.9	9	5.4 - 5.4	5.7[a], 24.6	0.0625 - 0.0625
	H. coronarium (Novel)	--	--	--	0.0	--	--

Table 6.4 (Contd.)

Beetle species	Plant species	N	Generation time (*T*) (Weeks)[a]	Age at first reproduction (Weeks)	Net reproduction rate (R_0)[a]	Life expectancies (e_x) (Weeks) [b]	Instantaneous population growth rate (*r*)[a]
C. dorsalis (Specialist)							
	C. malortieanus (Native)	28	14.0 - 23.4	10	3.9 - 13.3	8.9[a], 15.5	0.0976 - 0.1103
	C. speciosus (Exotic)	15	14.0 - 22.8	10	2.4 - 7.3	9.0[a], 22.5	0.0617 - 0.0871
C. placida (Specialist)							
	R. alpinia (Native)	40	15.1 - 24.4	11	1.6 - 4.8	10.3[a], 16.6	0.0324 - 0.0644
	A. purpurata (Exotic)	93	19.2 - 19.2	19	0.02 - 0.02	5.8[a], 15.6	0.2008 - 0.2008
	H. coronarium (Exotic)	--	--	--	0.0	--	--

[a] Values represent minimum-maximum parameter estimates. Minimum values assume that females don’t reproduce after their seventh week of life. Maximum values assume that female fecundity is contant from their seventh week of life until death (*see* Figure 6.4 for fecundity estimates).

[b] Life expectancy after larval eclosion [a] , Life expectancy after first reproduction



■ Native host
□ Novel host
Cephaloleia belti (Generalist)
A.
HL
HP
MV
MV=HP<HL
B.
HL
HP
MV
C.
HL=MV<HP
HL
MV
HP
Cephaloleia dilaticollis (Generalist)
D.
HC<AP=RA
RA
AP
HC
E.
RA
AP
HC
F.
HC<RA<AP
HC
RA
AP
Cephaloleia dorsalis (Specialist)
G.
CS<CM
CM
CS
H.
CM
CS
I.
CS=CM
CS
CM
Cephaloleia placida (Specialist)
J.
HC=AP<RA
RA
AP
HC
K.
***
RA
AP
HC
L.
HC<AP=RA
AP
HC
RA
Percent of larvae alive
Percent of pupae alive
Percent of adults alive
0.0
0.5
1.0
0
50
100
100
200
300
400
Days
Host plants
Days

FIGURE 6.1. Larval and pupal survival and adult longevity in *Cephaloleia* beetles expanding their diets from native to novel host plants. A - C. *Cephaloleia belti* (Generalist) in the native host *Heliconia latispatha* (HL) and the novel hosts *H. psittacorum* (HP) and *M. velutina* (MV). D – F. *Cephaloleia dilaticollis* (Generalist) in the native host *R. alpinia* (RA) and the novel hosts *A. purpurata* (AP) and *H. coronarium* (HC). G - I. *Cephaloleia dorsalis* (Specialist) in the native host *C. malortieanus* (CM) and the novel host *Ch. speciosus* (CS). J – L. *Cephaloleia placida* (Specialist) in the native host *R. alpinia* (RA) and the novel hosts *A. purpurata* (AP) and *H. coronarium* (HC). Differences among diets for larval survival and adult longevity $P < 0.05$. Pupae survival: *** $P < 0.001$.

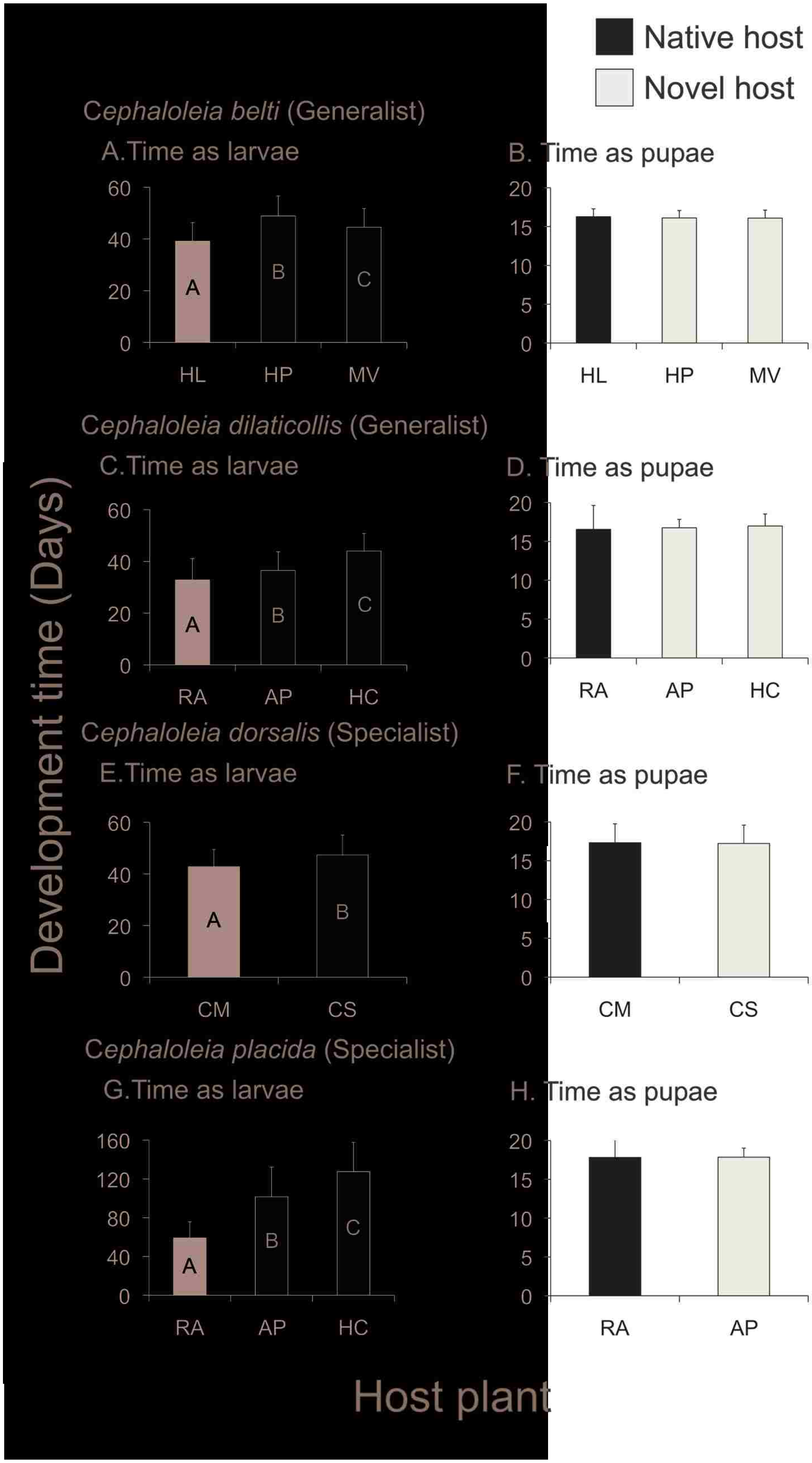

Native host
Novel host
Cephaloleia belti (Generalist)
A.Time as larvae
60
40
20
0
A
B
C
HL
HP
MV
B. Time as pupae
20
15
10
5
0
HL
HP
MV
Cephaloleia dilaticollis (Generalist)
C.Time as larvae
60
40
20
0
A
B
C
RA
AP
HC
D. Time as pupae
20
15
10
5
0
RA
AP
HC
Cephaloleia dorsalis (Specialist)
E.Time as larvae
60
40
20
0
A
B
CM
CS
F. Time as pupae
20
15
10
5
0
CM
CS
Cephaloleia placida (Specialist)
G.Time as larvae
160
120
80
40
0
A
B
C
RA
AP
HC
H. Time as pupae
20
15
10
5
0
RA
AP
Development time (Days)
Host plant

FIGURE 6.2. Effect of larval diet on larval development times (Mean ± SD) and time as a pupa. A - B. *Cephaloleia belti* (Generalist). C – D. *Cephaloleia dilaticollis* (Generalist). E - F. *Cephaloleia dorsalis* (Specialist). G – H. *Cephaloleia placida* (Specialist). Host plant abreviations as in Figure 6.1. Letters in the bars represent differences among treatments ($P < 0.05$).

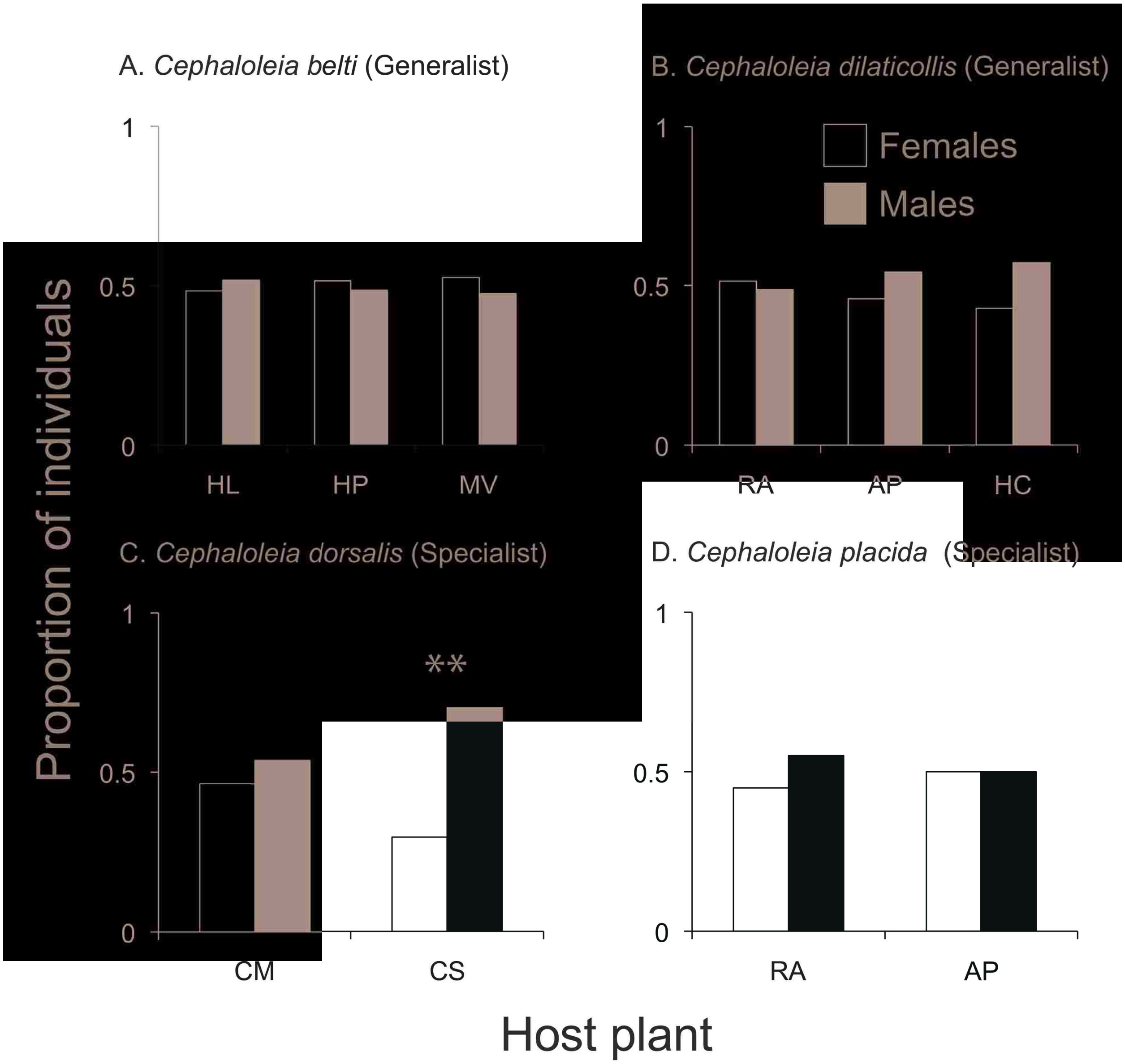


FIGURE 6.3. Sex ratio of adults raised in original or novel host plants. A. *Cephaloleia belti* (Generalist). B. *Cephaloleia dilaticollis* (Generalist). C. *Cephaloleia dorsalis* (Specialist). D. *Cephaloleia placida* (Specialist). Host plant legends as in Figure 6.1. ** P < 0.01.

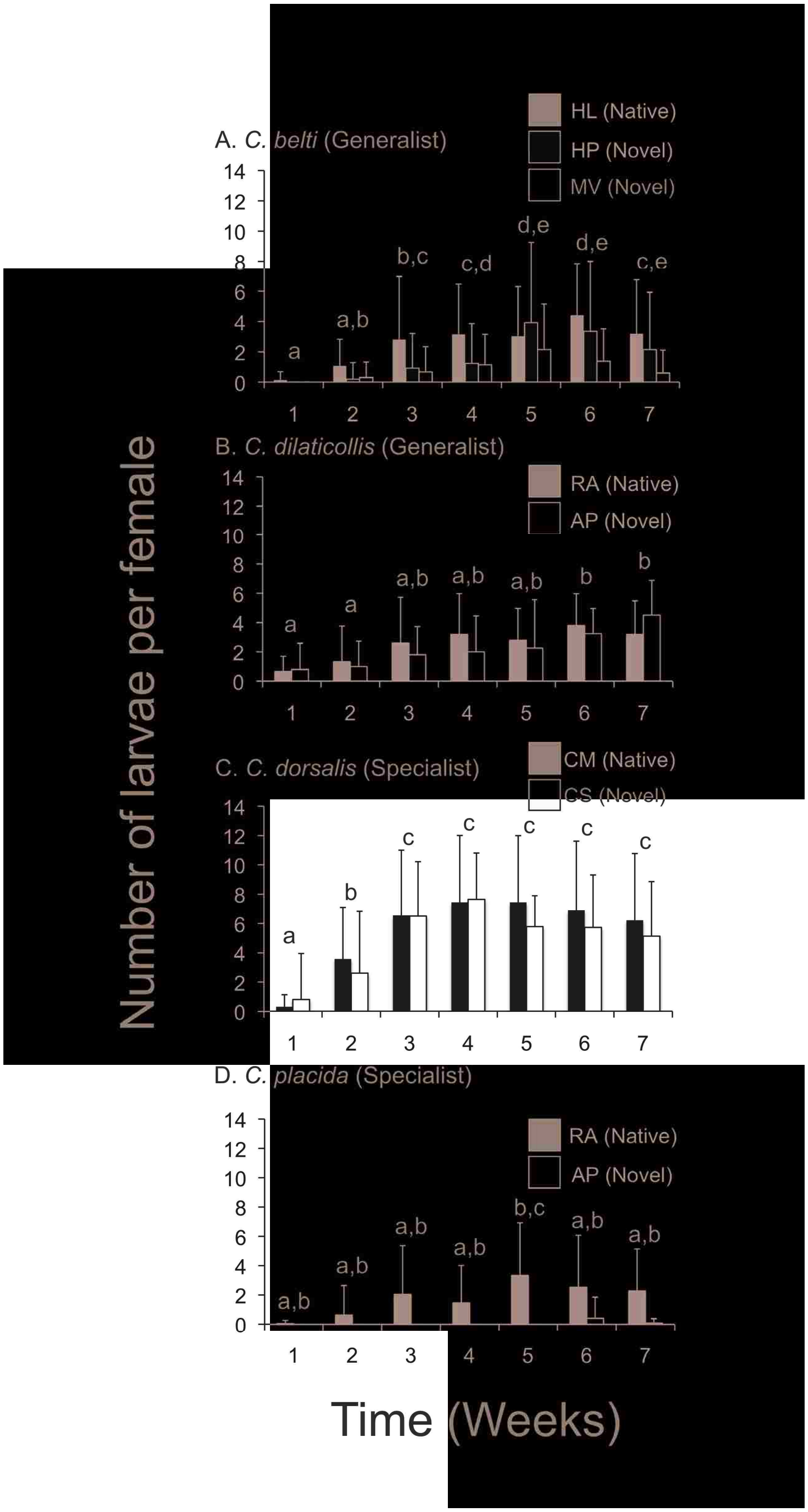


FIGURE 6.4. Fecundity of females in original and novel host plants. Host plant abreviations as in Figure 6.1. Letters on the bars group similar categories, $P < 0.05$.

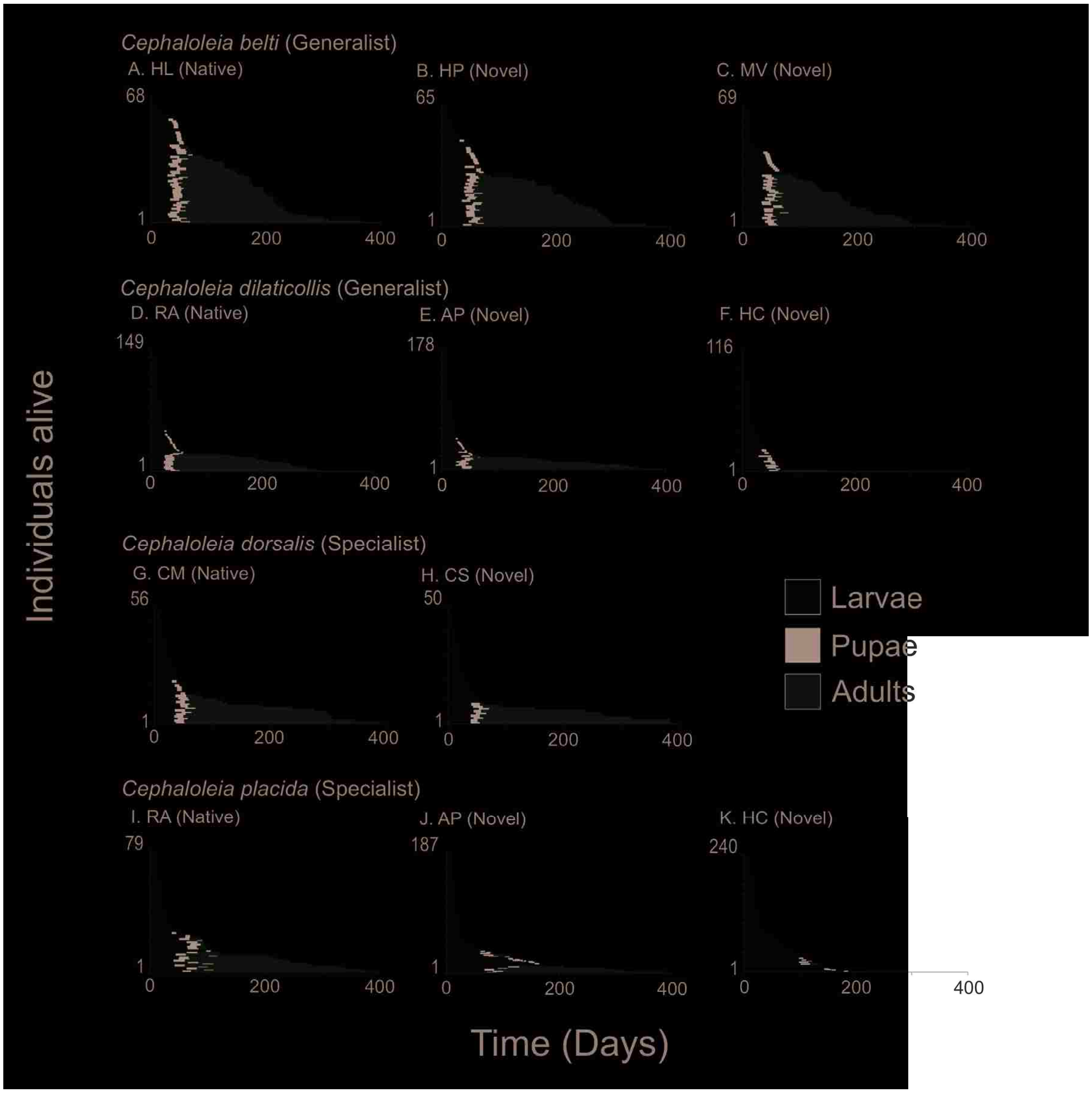


FIGURE 6.5. Event history diagrams for cohorts of rolled leaf beetles reared in original and novel host plants. A – C. *Cephaloleia belti*. D – F. *Cephaloleia dilaticollis.* G – H. *Cephaloleia dorsalis.* I- K. *Cephaloleia placida.* Each horizontal line portrays the lifecourse of an individual beetle. The length of each line is proportional to the individual's lifespan. Taken together, the bars provide the empirical survival function. Host plant abreviations as in Figure 6.1.

Chapter VII

Jack of all trades masters new hosts: positive genetic correlations in generalist and specialist *Cephaloleia* beetles expanding their diets to exotic host plants[6]

SUMMARY

In plant-herbivore interactions, the diets of most insect herbivores are specialized. A broadly accepted explanation for this widespread resource specialization is the "Jack of all trades master of none" principle. This principle proposes that genotypes with high performance in one host will perform poorly in other hosts. As a result, it is expected that specialization will be selected over generalization. A fundamental prediction of this principle is that the performances of genotypes in different hosts will be negatively correlated. In this study we explore cross-host correlations in performance for generalist and specialist insect herbivores that are currently undergoing an expansion in diets beyond their native hosts, to include new exotic host plants. One of the oldest and most conservative plant-herbivore associations is the interaction between beetles of the neotropical genus *Cephaloleia* and plants of the Zingiberales. At La Selva Biological Station, a tropical rainforest in Costa Rica, Central America, generalist and specialist *Cephaloleia* beetles are expanding their diets to paleotropical and South American Zingiberales. For two generalist and two specialist species of *Cephaloleia*, this study estimates genotype × host plant interactions, genetic correlations and heritabilities in the laboratory for: a. larval development and b. larval survival in native or novel hosts.

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[6] Coauthor: Carol C. Horvitz

Larvae of generalist and specialist *Cephaloleia* displayed similar responses when reared in native and novel host plants. Larval, pupal and adult sizes were reduced and development times increased for individuals reared on the novel hosts. Genotype × host plant interactions were not detected for any of the developmental traits or survival. All significant cross-host correlations were positive, not negative. These results represent very different ecological and evolutionary dynamics than those predicted by the "Jack of all trades master of none" principle. In this case high performance in the native host plant enhances the performance in exotic hosts promoting generalization.

BACKGROUND

Plants and their associated phytophagous insects are an important component of the biological diversity (Mitter et al. 1991). One of the processes involved in the generation of this outstanding diversity is the co-adaptation between plants and their insect herbivores (Ehrlich and Raven 1964, Janzen 1980, Futuyma and Slatkin 1983). A general pattern observed in plant-herbivore interactions is that the diets of most insect herbivores are specialized to one or a few host plants (Fox and Morrow 1981, Jaenike 1990, Thompson 1995, Novotny and Basset 2005). A broadly accepted explanation for this widespread resource specialization of insect herbivores is the "Jack of all trades master of none" principle (McArthur 1972, Futuyma and Moreno 1988, Via 1990, Futuyma et al. 1995). This principle proposes that there is a cost of adaptation to new hosts. Therefore there is a trade-off in performances between host plants. Genotypes displaying a high performance on a given host will perform poorly on other hosts.

This constraint of adaptation to multiple host plants implies that diet specialization will be selected over generalization. This principle is a central assumption of several models for the evolution of specialization (Barbosa 1988, Futuyma and Moreno 1988, Agrawal 2007). A fundamental prediction of this principle is that the performance of herbivore genotypes on different hosts will be negatively correlated (Futuyma and Moreno 1988, Ueno et al. 2003, Agosta and Klemens 2009).

Negative genetic correlations in cross-host performance were reported for some insect herbivores (Via 1984, Mackenzie 1996, Tilmon et al. 1998). However, most studies report positive or no correlation between genotype performance on different hosts (Futuyma and Philippi 1987, Ueno *et al.* 2003, Futuyma 2008).

An interpretation of an absence of correlation in cross-host performance is that genotypes performance in a given host is not constrained by their performance in other host plants. In the case of positive correlations, genotypes that perform well in a host will also performance well in other host plants. Positive correlations in cross-host performance represent very different ecological and evolutionary dynamics than those predicted by the "Jack of all trades master of none" principle. With positive genetic correlations, high performance on one host plant enhances the performance on other plants, promoting generalization.

Although there has been considerable focus on specialist herbivores, some insects are generalists, experiencing multiple host plants over their lifetime (Singer 2001). The use of several environments (in this case multiple host plants), may promote the evolution of composite generalized diets, where multiple genotypes display different performance among hosts, or the evolution of plastic generalist genotypes that can survive and reproduce successfully in several host plants (Powell 1971, Powell and Wistrand 1978, Fox and Morrow 1981, Hawthorne 1997, Agrawal 2001, 2007). Therefore it is possible that relative performance across host plants differ between generalist and specialist herbivores. For example, composite generalization may translate into genotype × host plant interactions and negative correlations in performance across hosts. However, if generalist genotypes display adaptive plasticity that translates into similar performance among hosts, we would expect to find non-significant or positive correlations.

One of the criticisms of some studies that repor the absence of negative correlations in performance across hosts is that genotype responses were usually measured on host plants that had been interacting with the particular herbivore for several generations (Fry 1993, Agosta and Klemens 2008). Therefore, the expected trade-offs in host use may be reduced or no longer present in the populations if herbivore have previously adapted to the host plants. To understand differential performance of genotypes on different host plants, one must study recently assembled plant-herbivore interactions. New plant-herbivore interactions between native insect herbivores and recently introduced exotic plants would be good subjects for such a study.

One of the oldest and most conservative plant-herbivore associations is the interaction between beetles of the neotropical genus *Cephaloleia* (Chrysomelidae: Cassidinae) and plants of the Zingiberales. *Cephaloleia* beetles and neotropical gingers evolved in the neotropics for the last 40-60 MY in isolation from paleotropical Zingiberales (Wilf et al. 2000, McKenna and Farrell 2005, McKenna and Farrell 2006, García-Robledo and Staines 2008).

At La Selva Biological Station (Costa Rica, Central America), the tropical rain forest where we performed this research, 40 species of *Cephaloleia* are associated with 43 native plants of the Zingiberales (Staines 1996). In the last decade five paleotropical and one South American species of exotic Zingiberales have naturalized at the La Selva Biological Station. Seven *Cephaloleia* species with contrasting diet breadths are currently expanding their diets to these exotic Zingiberales, generating 16 novel plant-herbivore interactions (Chapter I).

These novel plant-herbivore associations provide an opportunity to study the differential performance of herbivore genotypes on novel host plants. In the present study, we use a quantitative genetic approach to investigate the genetic architecture of larval development and survival on native and novel hosts. To explore the effects of insect herbivore diet breadth on genotype responses on native and novel hosts, we selected two generalist and two specialist *Cephaloleia* beetles currently expanding their diets to four paleotropical and one south american Zingiberales (Table 7.1). The objectives of this research are (1) to determine the effects of using novel hosts as a food sources on larval development and survival. (2) to estimate genotype × environment interactions, heritabilities and genetic correlations of morphology and survival. In this study genotype is a mix of half and full sibling family groups, and environment is host plant species. Finally, we discuss if genotypes in recently assembled plant-herbivore interactions are constrained in their use of novel hosts, display negative or positive correlations in cross-host performance.

METHODS

STUDY SITE AND SPECIES — We conducted this research at La Selva Biological Station (hereafter La Selva) from August 2005 to March 2009. La Selva is a tropical rain forest in Costa Rica, Central America (10º26'N, 83º59'W). In this study we selected four *Cephaloleia* beetle species with contrasting diet breadths as study models (Table 7.1). At La Selva, *Cephaloleia belti* is the species with the broadest diet breadth, feeding on 15 species from three families of Zingiberales (García-Robledo et al. In press). The beetle *Cephaloleia dilaticollis* is also a generalist, feeding on ten species from three families of Zingiberales (García-Robledo et al. In press).

We also selected two specialists. *Cephaloleia dorsalis* is a specialist on the family Costaceae. At La Selva *C. dorsalis* was recorded feeding on four species of the genus *Costus*. *Cephaloleia placida* is a specialist on the family Zingiberaceae. At La Selva this species feeds on two species of the genus *Renealmia* (García-Robledo *et al.* in press).

Adults of these *Cephaloleia* species feed on the leaf tissue of the young rolled leaves of their host plants. In contrast, larvae feed on the leaf tissue of expanded leaves (García-Robledo *et al.* In press). These four beetle species are currently expanding their diets at La Selva by including naturalized exotic hosts from India, the Malay Peninsula, the Pacific Islands and South America into their diets (Table 7.1).

LARVAL DEVELOPMENT AND SURVIVAL ON NATIVE AND NOVEL HOSTS — To determine if generalist vs specialist *Cephaloleia* differ in the degree to which their larvae are affected by switching to a novel host, we performed the following experiment. We collected males and females of each species from the native host species on which it is most frequently encountered in the field at La Selva Biological Station (García-Robledo *et al*. in press; Table 7.1). Mating couples were placed in separate 17 X 15 X 5 cm containers and fed *ad libitum* with young leaf tissue from their native host plants. In each container we also included four 10 X 10 squares of fully expanded leaf from the native host as oviposition substrate. Leaves were changed every 48 h (Number of females: $N_{C.belti} = 38$, $N_{C.dilaticollis} = 32$, $N_{C.dorsalis} = 37$, $N_{C.placida} = 42$)**.** Eggs were carefully removed from the leaf surface and placed in containers lined with moist filter paper. We recorded the female from which each egg was obtained. After eclosion, larvae obtained from each female were randomly assigned to one of the following diets: leaf tissue from the native host or leaf tissue from the novel host plant (Table 7.1). These records were used in further quantitative genetics analyses (*see* next section).

Each larva was placed in an individual container lined with moist filter paper. Larvae were fed every 48 h with two 3.5 cm diameter disks of leaf tissue. Larvae were reared at a mean temperature of 27°C and a light regime of 12 h. light 12 h. darkness (sample sizes for the number of larvae reared on each host plant are given in Table 7.2)

To measure larval growth rates, we measured the length of each newborn larvae from the tip of the head to the tip of the abdomen. We performed two additional length measurements during larval development, one at the mean estimated time of transition on native hosts from first to second instar (*i.e* the end of the first instar) and the other at the time of transition from the second instar to the pupation on native hosts (*i.e.* the end of the second instar) (*see* estimated transition times for each species in Figure 7.1 and García-Robledo et al. in press).

We measured larval lengths using a digital camera (Diagnostic Instruments Inc. Model 3.2.0) attached to a stereoscope (Leica MZ 12s). Lengths of the larvae were estimated on the digital images at an accuracy of 10^{-2} mm, using the program Spot V.3.5.8 (Diagnostic Instruments Inc. Sterling Heights, MI). Data were log-transformed. Differences in length among larvae were tested with one-way ANOVA's.

Pupal lengths and weights were measured on the day of pupation. Weight was measured using an analytic balance Scientech SA 40 with a precision of 10^{-4} g and log-transformed. Adult lengths and weights were measured on the day of adult emergence and log-transformed. For each individual we recorded the time from larval eclosion to pupation, and from pupation to adult emergence. Differences in pupal length, pupal weight, adult length, adult weight and development times of individuals in the native vs novel hosts were tested with one-way ANOVA's, when there were more than two host plants and by Welch's t-tests when there were only two hosts being compared (R-Development-Core-Team 2009).

To estimate larval survival on native and novel host plants, we monitored each larva every 48 h until death or pupation. Differences in larval mortality between native and novel hosts were determined by Cox proportional hazard survival analyses. Cox proportional hazard examines the effects of covariates or continuous independent variables on the risk of death at different ages. It is particularly useful for making contrasts based on covariate values without being particularly concerned with the shape of the survival function for the group designated as the base line pattern. Thus the risk of death is modeled for individuals based on the values of their covariates, relative to a baseline pattern. This type of analysis is a regression model similar to life table survival analysis like Kaplan-Meier analyses. Cox proportional hazard models can include interactions among multiple covariates, allowing to explore the genotype × environment interaction effects on survival. The model is specified as:

$$h_i(t) = h_0(t)\exp(\beta_1 X_{i1} + \beta_{21} X_{i2} + ... + \beta_k X_{ik}$$

Where $h_o(t)$ is the baseline or reference hazar function that is changed by the values. The covariates X and regression coefficients β's. The values of the covariates X_{ik}'s varies among individuals (i) essentially this type of analysis considers the relative likelihood of death at a given time among indiviuals based on the values of their covariances, in our case based on genetic family and host plant (Fox 2001).

QUANTITATIVE GENETIC ANALYSES — During the development and survival experiments we recorded the female from which each larva was obtained. The offspring of each female (here after a genetic family) represents an unknown mix of half and full siblings because the females were collected in the field, potentially mating with more than one male. We know that they are at least half sibs. The heritability estimates reported in the following analyses include both additive and non-additive genetic variance such as dominance, epistatic and maternal effects. Therefore these estimates represent broad-sense heritabilities (Table 7.5).

For the following analyses we grouped the data obtained for all individuals during the experiments testing for differences in development and survival in native and novel hosts by their respective genetic families. In cases where genetic families had no individuals surviving in one of the host plants, genetic families were removed from the analyses (*see* sample size in Figure 7.5).

Family x host interactions of development and survival in native and novel hosts

To determine if generalist and specialist *Cephaloleia* species have genetic variation in developmental traits, we estimated the proportion of variance due to family and compared the mean performance of genetic families for the following developmental traits in native and novel hosts: a. length of larvae at instar 1, b. larval length at instar 2, c. pupa length, d. adult length, e. pupa weight, f. adult weight, g. development time from larval eclosion to pupation and h. development time from pupation to adult emergence (Table 7.3). Larvae, pupae and adult lengths were log-transformed. For each developmental trait, differences among families were estimated with an ANOVA model (Table 7.3).

Family x host interactions were estimated for each developmental trait. Analyses were performed used linear mixed-effects models, employing restricted maximum likelihood (REML) (R-Development-Core-Team 2009)**.** Only larvae, pupae and adult lengths were log-transformed. The diet assigned to each larva (native vs novel hosts) was included as a fixed factor and genetic family as a random factor.

To determine if generalist and specialist *Cephaloleia* species reared on native and novel hosts display genetic variation in survival, we performed the following analyses. Differences in survival among families were tested for each beetle species with mixed Cox proportional hazard models (Package Survival, R-Development-Core-Team, 2009). The model included larval diet as a fixed factor and genetic families as a random factor (*see* sample size in Table 7.4). We determined family x host plant interactions, by comparing the survival models with or without the family x host plant interaction term. Comparisons between models were performed using likelihood-ratio tests.

Finally, we estimated the broad-sense heritability (H2) for each trait measured in the laboratory. These estimates represent the proportion of phenotypic variation that is attributable to genetic variation (in a broad sense) among individuals.

Genetic correlations of development and survival in native and novel hosts

For each *Cephaloleia* species, we estimated genetic correlations between members of the same genetic family reared in either the native of a novel host plants. Genetic correlations were estimated for the following traits: a. larval length at instar 1, b. larval length at instar 2, c. pupa length, d. adult length, e. pupa weight, f. adult weight, g. development time from larval eclosion to pupation, h. development time from pupation to adult emergence and i. larval survival (*see* sample size in Figure 7.5). In all *Cephaloleia* species, larval, pupal and adult lengths were log-transformed. Survival proportions were arcsin transformed. Correlations in relative performance among host plants were estimated using Pearson's product moment correlations.

RESULTS

LARVAL DEVELOPMENT IN NATIVE AND NOVEL HOSTS

Larvae pupae and adult length

In the four species of *Cephaloleia*, larvae, pupae and adults were longer in the native than in the novel host plants. In the generalist beetle *Cephaloleia belti,* larvae, pupae and adults were longer in the native host *H. latispatha* than in the novel hosts (Figure 7.1A, Table 7.2). Larvae in first instar, were longer in the novel host *M. velutina* than in the novel host *H. psittacorum*. However, individuals in second larval instar, pupa and adults were longer when reared in the novel host *H. psittacorum* than when reared in the novel host *M. velutina.* (Figure 7.1A, Table 7.2).

Larvae, pupae and adults of the generalist beetle *C. dilaticollis* reared in the native host *R. alpinia* and the novel host *A. purpurata* were longer than individuals reared in the novel host *H. coronarium* (Figure 7.1B, Table 7.2).

Larvae, pupae and adults of the specialist *C. dorsalis* reared in the native host *C. malortieanus* were longer than individuals reared in the novel host *Ch. speciosus* (Figure 7.1C, Table 7.2). In the specialist beetle *C. placida,* larvae and pupae were longer in larvae reared in the native host than in larvae reared in the novel hosts. Larval and pupal mortality was very high for *C. placida* larvae reared in the novel host *H. coronarium;* no adults emerged from pupae reared in this novel host (Figure 7.1D, Table 7.2). Adults were longer when reared in the native host *R. alpinia* than in the novel host *A. purpurata* (Table 7.2).

Pupal and adult weight

In general, pupae and adults were heavier when reared in the native hosts. Pupae and adults of the generalist beetle *C. belti* were heavier when reared in the native host plant *H. latispatha* than individuals reared in the novel hosts *H. psittacorum* and *M. velutina* (Figure 7.2A, Table 7.2). Pupae and adults reared in the novel host *H. psittacorum* displayed the lowest weights (Figure 7.2A, Table 7.2).

Pupae and adults of the generalist beetle *C. dilaticollis* were heavier when reared in the native host plant *R. alpinia* than individuals reared in the novel hosts *A. purpurata* and *H. coronarium* (Figure 7.2B, Table 7.2). Pupae and adults reared in the novel host *H. coronarium* displayed the lowest weights (Figure 7.2B, Table 7.2).

Pupae and adults of the specialist beetle *C. dorsalis* were heavier when reared in the native host plant *C. malortieanus* than individuals reared in the novel host *Ch. speciosus* (Figure 7.2C, Table 7.2). Pupae of the specialist beetle *C. placida* reared in the native host *R. alpinia* were heavier than individuals reared in the novel hosts *A. purpurata* and *H. coronarium.* Pupal mortality was very high for larvae and pupae of *C. placida* in the novel host *H. coronarium;* no adults emerged from pupae reared in this novel host (Figure 7.2D, Table 7.2). Adults were heavier when reared in the native host than in the novel host *A. purpurata.*

Development time

In general, development times from egg eclosion to pupation were shorter in individuals reared in the native than in individuals reared in the novel host plants (Figure 7.3). However, development time from pupation to adult emergence was not affected by larval diet (Figure 7.3, Table 7.2)

LARVAL SURVIVAL IN NATIVE AND NOVEL HOSTS — In the four species of *Cephaloleia*, larval survival was usually higher in the native than in the novel host plants (Figure 7.4, Table 7.4). Larval survival of the generalist *C. belti* was higher in the native host *H. latispatha,* and equivalent in the novel hosts *H. psittacorum* and *M. velutina* (Figure 7.4A, Table 7.4). The generalist *C. dilaticollis* displays equivalent survival in the native host *R. alpinia* and the novel host *A. purpurata* (Figure 7.4B, Table 7.4). Mortality of *C. dilaticollis* larvae in *H. coronarium* was high (Figure 7.4B, Table 7.4).

Larval survival of the specialist *C. dorsalis* was higher in the native host *C. malortieanus* than in the novel host *Ch. speciosus* (Figure 7.4C, Table 7.4). Larval survival of the specialist *C. placida* was higher in the novel host *R. alpinia* than in the novel hosts *A. purpurata* and *H. coronarium* (Figure 7.4C, Table 7.4). Mortality of larvae reared in the novel host *H. coronarium* was high (Figure 7.4C, Table 7.4).

QUANTITATIVE GENETIC ANALYSES

Family x host interactions of development and survival in native and novel hosts

In general, we detected differences among families for most of the developmental traits measured in the laboratory. However, for most of the traits, we did not detect family x host plant interactions (Table 7.3, Table 7.5). Families of the generalist *C. belti* displayed differences in mean larval, pupal and adult size and weight as well as in development times (Table 7.3, Table 7.5). We only detected differences in family x host interactions for the larval length measured during the first instar (Table 7.3, Table 7.5).

In the generalist *C. dilaticollis,* pupal and adult mortality were high in the novel host *H. coronarium* (Figure 7.4B, Table 7.5). For this reason, we only explored genetic variation and family x host plant interactions for families reared in the native host *R. alpinia* and the novel host *A. purpurata* for the following traits: pupal and adult lengths, pupal and adult weights development times (Table 7.3, Table 7.5). Families of the generalist *C. dilaticollis* displayed differences in mean larval and pupal length, pupal weight and development time from egg eclosion to pupation (Table 7.3, Table 7.5). The length and weight of adult *C. dilaticollis* and the development time from pupation to adult emergence were similar among families. Family x host interactions were not significant (Table 7.3, Table 7.5).

Families of the specialist *C. dorsalis* displayed different larval and adult lengths and weights. Families of *C. dorsalis* displayed equivalent development times from larval eclosion to pupation and from pupation to adult emergence (Table 7.3, Table 7.5). Family x host interactions were not significant (Table 7.3, Table 7.5).

Families of the specialist *C. placida* displayed differences in larval length (Table 7.3, Table 7.5). Larval and pupal mortality were very high in the novel host plants (Figure 7.4D). Therefore, differences among families in size, weight and development times were not measured for pupae and adults of *C. placida* (Table 7.3, Table 7.5).

For the four species of *Cephaloleia* beetles, larval survival is different among families (Table 7.4). We did not detect family x host interactions for larval survival in any of the beetle species (Table 7.4).

Genetic correlations of development and survival in native and novel hosts

In general, we did not record negative correlations for the developmental traits or survival in families reared in native or novel hosts. In the four *Cephaloleia* species, all correlations were positive or not significant (Figure 7.5).

In the generalist *C. belti,* nine correlations were positive, and 18 not significant (Figure 7.5A). In the generalist *C. dilaticollis,* pupal and adult mortality were high in the novel host *H. coronarium* (Figure 7.4B). For this reason in some of the traits we only performed correlations between the native host *R. alpinia* and the novel host *A. purpurata*

(Figure 7.5B). In *C. dilaticollis*, two correlations were positive and 13 were not significant (Figure 7.5B).

In the specialist *C. dorsalis* we recorded three positive and six not significant correlations (Figure 7.5C). In the specialist *C. placida,* larval mortality was high in both novel hosts (Figure 7.4D). For this reason we only performed correlations for the traits directly associated with larval development and survival (Figure 7.5D). In this species we recorded three positive and six not significant correlations.

DISCUSSION

Our results suggest no evident physiological advantage for generalist *Cephaloleia* species over specialists colonizing novel hosts. Development time was extended and survival was reduced for both generalist and specialist species in the novel hosts. As a result, the mean phenotypes in the novel hosts changed. Adults were smaller in the novel than in the native hosts. The high mortalities of larvae in the novel hosts show that novel hosts are challenging and individuals expanding their diets to novel hosts will most likely face strong selection. This is not a surprising result, as the fitness of insect herbivores is usually reduced in novel hosts during early colonization (Futuyma and Moreno 1988, Scheirs et al. 2000).

We detected substantial genetic variation in both generalist and specialist herbivores for both developmental traits and larval survival. Differences in performance among families are the product of an unknown combination of additive and non-additive genetic vatiation (Falconer and Mackay 1996). A virtual absence of genotype × host-plant interactions suggests that the response of genotypes to novel hosts is similar.

Similar to the results of most previous studies, we found no evidence for the negative correlation in cross-host performance predicted by the “Jack of all trades master of none” principle (Falconer and Mackay 1996). Three arguments trying to explain the absence of negative correlation in cross-host performance are that: a. genetic trade-offs can be obscured by some environmental effects such as e.g. maternal effects. b. genetic correlations are an inadequate method to estimate trade-offs and c. native trade-offs in host use are quickly ameliorated by selection (Rausher 1988, Fry 1993).

Our estimates of broad-sense heritability might be obscured by environmental effects (Falconer and Mackay 1996). However, it is unlikely that selection already reduced the native trade-offs in *Cephaloleia* beetles expanding their diets to exotic Zingiberales. It is intriguing that although we measured several traits among multiple novel host plants and for four herbivore species with contrasting diet breadths, we found absolutely no evidence for trade-offs in host use. Maybe the most parsimonious argument based solely in the current evidence and not in the theoretical expectations of evolutionary models based on trade-offs, is that negative correlations in cross-host performance are not a major component of the genetic architecture of insect herbivores.

In this study we found that significant correlation in cross-host performance are always positive. Positive genetic correlations in performance represent ecological and evolutionary dynamics opposite to those predicted by the “Jack of all trades master of none” principle. In this case, high performance in a native host plant increases the probability of a better performance in novel hosts, promoting generalization.

In conclusion, our results support the idea that the genetic architecture of insect herbivores does not promote specialization as suggested by trade-off theory. On the contrary, the genetic architecture of *Cephaloleia* beetles may facilitate the colonization of novel hosts, either exotic Zingiberales from the Paleotropics or the neotropical gingers they evolved with for the last 40-60 MY.

Table 7.1. *Cephaloleia* beetles and the native and exotic host plants selected to quantify changes in developmental and survival traits and their broad-sense heritabilities (H^2) in genetic families reared in the native and novel host plants.

Beetle species	**Native host plant**	**Novel host plants**	**Origin of novel hosts**
Cephaloleia belti (Generalist)			
	Heliconia latispatha (Heliconiaceae)	*Heliconia psittacorum* (Heliconiaceae)	Caribbean, northern South America
		Musa velutina (Musaceae)	India
Cephaloleia dilaticollis (Generalist)			
	Renealmia alpinia (Zingiberaceae)	*Alpinia purpurata* (Zingiberaceae)	Pacific islands
		Hedychium coronarium (Zingiberaceae)	Eastern India
Cephaloleia dorsalis (Specialist)			
	Costus malorteanus (Costaceae)	*Cheilocostus speciosus* (Costaceae)	Malay Peninsula of Southeast Asia
Cephaloleia placida (Specialist)			
	Renealmia alpinia (Zingiberaceae)	*Alpinia purpurata* (Zingiberaceae)	Pacific islands
		Hedychium coronarium (Zingiberaceae)	Eastern India

Table 7.2. ANOVA and Welch two-sample T test results comparing the expression of developmental traits in larvae of *Cephaloleia* reared in native and novel host plants. Host plant abbreviations as in Figure 1.

Beetle species	Traits	Sample size			DF	F	P
Cephaloleia belti (Generalist)		HL	HP	MV			
	Length (mm)						
	Newborn larvae	348	392	403	2	0.60	0.55
	Larvae instar 1	292	295	278	2	355.19	**< 0.0001**
	Larvae instar 2	254	272	252	2	47.13	**< 0.0001**
	Pupae	278	250	228	2	75.033	**< 0.0001**
	Adults	210	173	163	2	31.64	**< 0.0001**
	Weight (mg)						
	Pupae	210	173	162	2	59.21	**< 0.0001**
	Adults	210	173	162	2	59.20	**< 0.0001**
	Development time (d)						
	Time as a larva	278	250	228	2	115.17	**< 0.0001**
	Time as a pupa	210	173	163	2	1.99	0.1372

Table 7.2 (Contd.)

Beetle species	Traits	Sample size			DF	F	P
Cephaloleia dilaticollis (Generalist)		RA	AP	HC			
	Length (mm)						
	Newborn larvae	566	556	215	2	2.9	0.05
	Larvae instar 1	253	255	61	2	42.03	**< 0.0001**
	Larvae instar 2	100	126	39	2	9.46	**< 0,0001**
	Pupae	142	129	27	2	14.94	**< 0.0001**
	Adults	74	75	10	2	16.14	**< 0.0001**
	Weight (mg)						
	Pupae	142	129	27	2	51.03	**< 0.0001**
	Adults	74	75	10	2	25.24	**< 0.0001**
	Development time (d)						
	Time as a larva	143	130	27	2	26.35	**< 0.0001**
	Time as a pupa	74	75	11	2	0.26	0.77

Table 7.2 (Contd.)

Beetle species	Traits	Sample size		DF	t	P
Cephaloleia dorsalis (Specialist)		CM	CS			
	Length (mm)					
	Newborn larvae	338	323	658.63	-1.24	0.2153
	Larvae instar 1	175	130	264.74	7.88	**<0.0001**
	Larvae instar 2	74	77	131.65	4.03	**<0.0001**
	Pupae	116	78	164.7	2.77	**0.0062**
	Adults	86	58	129.18	2.86	**0.0049**
	Weight (mg)					
	Pupae	116	78	170.84	4.43	**<0.0001**
	Adults	85	58	117.08	4.07	**<0.0001**
	Development time (d)					
	Time as a larva	115	80	153.88	-4.26	**<0.0001**
	Time as a pupa	84	58	137.49	0.8445	0.3999

Table 7.2 (Contd.)

Beetle species	Traits	Sample size			DF	Statistic	P
		RA	AP	HC			
Cephaloleia placida (Specialist)							
	Length (mm)						
	Newborn larvae	272	220	240	2	F = 0.71	0.4893
	Larvae instar 1	109	49	82	2	F = 179.45	**< 0.0001**
	Larvae instar 2	39	39	63	2	F = 98.78	**< 0.0001**
	Pupae	91	26	9	2	F = 45.40	**< 0.0001**
	Adults	70	14	--	1	t = -2.54	**0.021**
	Weight (mg)						
	Pupae	91	26	9	2	F = 36.89	**< 0.0001**
	Adults	70	14	--	1	t = -3.96	**< 0.0001**
	Development time (d)						
	Time as a larva	91	26	9	2	F = 72.92	**< 0.0001**
	Time as a pupa	71	14	--	1	t = 0.01	0.922

Table 7.3. ANOVA table for the comparison among genetic families and genotype × environment interactions for larval developmental traits expresed by larvae of *Cephaloleia* in native and novel host plants.

		Herbivore species											
		Cephaloleia belti (Generalist)			*Cephaloleia dilaticollis* (Generalist)			*Cephaloleia dorsalis* (Specialist)			*Cephaloleia placida* (Specialist)		
Trait	Factor	DF (num/den)	F	P	DF (num/den)	F	P	DF (num/den)	F	`P	DF (num/den)	F	P
Length (mm)													
Instar 1													
	Host	2/756	370.95	**<0.0001**	2/422	32.64	**<0.0001**	1/232	62.68	**<0.0001**	1/110	189.7	**< 0.0001**
	Family	34	2.17	**0.0002**	22	1.94	**0.0069**	31	2.29	**<0.0001**	20	2.38	**0.0019**
	Family X Host	68/756	1.40	**0.0218**	44/422	0.82	0.7852	31/232	1.08	0.3551	60/110	0.73	0.9054
Instar 2													
	Host	2/669	46.49	**<0.0001**	2/155	8.19	**<0.0001**	1/89	18.37	**<0.0001**			
	Family	34	48.94	**0.0003**	17	3.43	**<0.0001**	24	2.02	**0.0094**	8	2.69	**0.0197**
	Family X Host	68/669	1.22	0.1188	34/155	0.95	0.5558	24/89	1.49	0.0928	24/28	1.53	0.1405
Pupae													
	Host	2/647	81.27	**<0.0001**	1/218	1.02	0.3139	1/127	7.00	**0.0092**			
	Family	34	81.84	**<0.0001**	23	1.77	**0.0193**	24	2.12	**0.004**	--	--	--
	Family X Host	68/647	0.98	0.535	23/218	0.59	0.9333	24/127	1.39	0.1262	--	--	--
Adults													
	Host	2/438	32.71	**<0.0001**	1/102	7.52	**0.0072**	1/83	6.16	**0.0151**			
	Family	30	1.89	**0.0035**	21	0.94	0.5443	18	3.33	**<0.0001**	--	--	--
	Family X Host	60/438	8.84	0.7976	21/102	0.91	0.5785	18/83	1.62	0.073	--	--	--

Table 7.3 (Contd.)

		Herbivore species											
		Cephaloleia belti (Generalist)			*Cephaloleia dilaticollis* (Generalist)			*Cephaloleia dorsalis* (Specialist)			*Cephaloleia placida* (Specialist)		
Trait	Factor	DF (num/den)	F	P	DF (num/den)	F	P	DF (num/den)	F	P	DF (num/den)	F	P
Weight (mg)													
Pupae													
	Host	2/647	79.53	**<0.0001**	1/217	20.24	**< 0.0001**	1/127	19.97	**<0.0001**	--	--	--
	Family	34	3.26	**<0.0001**	22	1.94	**0.0089**	24	1.91	**0.0118**	--	--	--
	Family X Host	68/647	1.13	0.2302	22/217	0.72	0.8148	24/127	1.14	0.375	--	--	--
Adults													
	Host	2/437	63.50	**<0.0001**	1/102	18.00	**< 0.0001**	1/82	17.54	**<0.0001**			
	Family	30	2.61	**<0.0001**	21	1.08	0.3776	18	3.47	**<0.0001**	--	--	--
	Family X Host	60/437	0.86	0.7601	21/102	0.98	0.4957	18/82	1.55	0.0934	--	--	--
Development time (d)													
Egg to pupa													
	Host	2/647	129.83	**<0.0001**	1/220	17.85	**< 0.0001**	1/107	18.32	**<0.0001**			
	Family	34	3.90	**<0.0001**	23	1.45	**0.0885**	23	1.55	0.0662	--	--	--
	Family X Host	68/647	1.17	0.1731	23/220	0.75	0.7919	46/107	0.66	0.9438	--	--	--
Pupa to adult													
	Host	2/438	0.98	0.3765	1/102	0.61	0.4356	1/66	0.08	0.7730			
	Family	30	1.92	**0.0029**	21	0.43	0.986	17	0.91	0.5695	--	--	--
	Family X Host	60/438	1.28	0.0881	21/102	0.53	0.9504	34/66	0.27	0.9999	--	--	--

Table 7.4. Cox proportional hazard analyses for differences in larval survival in *Cephaloleia* beetles reared in native and novel host plants. N = the number of genetic families.

Beetle species		Log-likelihood	X^2	N	P
Cephaloleia belti (Generalist)					
	Host plant	-2501.1	35.21	36	**<0.0001**
	Family	-2492.0	18.11	36	**<0.0001**
	[1]Family x Host	-2492.04; -2492.29	0.49	36	0.78
Cephaloleia dilaticollis (Generalist)					
	Host plant	-6719.5	14.04	30	**0.0008**
	Family	-6685.3	68.34	30	**<0.0001**
	[1]Family x Host	-6685.3; -6691.6	12.49	30	**0.0019**
Cephaloleia dorsalis (Specialist)					
	Host plant	-2728.6	5.44	35	**0.0196**
	Family	-2711.6	33.99	35	**<0.0001**
	[1]Family x Host	-2711.6; -2712.4	1.53	35	0.2158
Cephaloleia placida (Specialist)					
	Host plant	-3404.4	17.352	38	**0.0002**
	Family	-3361	86.83	38	**<0.0001**
	[1]Family x Host	-3361.0; -3361.9	1.87	38	0.3924

[1] Log-likelihoods for model comparisons between the model without Family X Host interaction and the model including the interaction term.

Table 7.5. Broad-sense heritability (H^2) of larval traits in generalist and specialist *Cephaloleia* beetles.

	Broad-sense heritability (H^2)			
Traits	*C. belti*	*C. dilaticollis*	*C. dorsalis*	*C. placida*
Length (mm)				
Larvae instar 1	0.22	0.25	0.43	0.44
Larvae instar 2	0.24	0.38	0.56	0.50
Pupae	0.25	0.39	0.50	--
Adults	0.29	0.52	0.53	--
Weight (mg)				
Pupae	0.25	0.38	0.50	--
Adults	0.29	0.52	0.53	--
Development time (d)				
Time as a larva	0.25	0.39	0.49	--
Time as a pupa	0.29	0.52	0.52	--

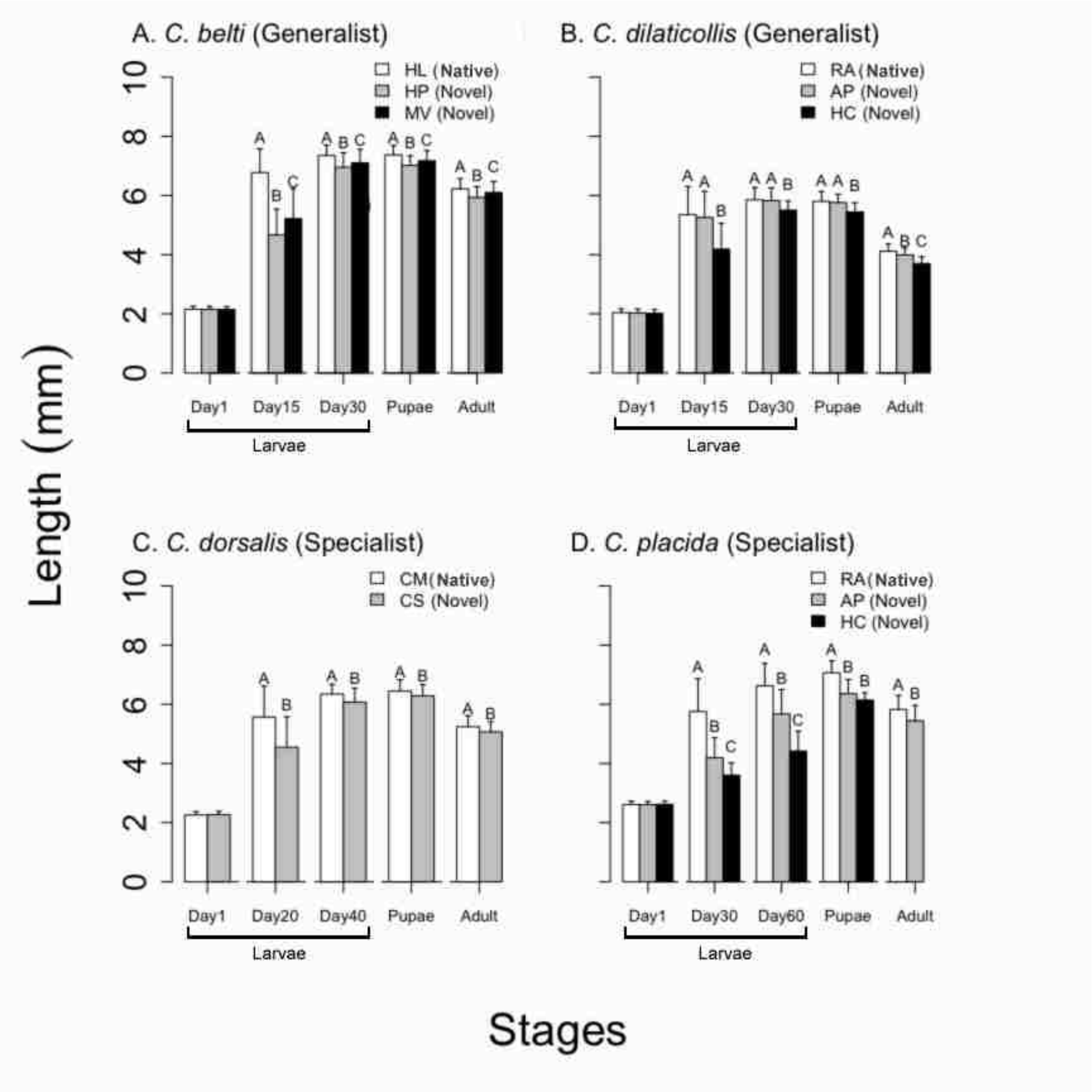

Figure 7.1. Length (Mean ± SD) of larvae, pupae and adults of generalist and specialist *Cephaloleia* beetles reared in native and novel host plants. Larval lengths were measured after hatching, at instar 1, and the end of instar 2 development times. A. *Cephaloleia belti*. HL: *Heliconia Latispatha*, HP: *Heliconia psittacorum*, MV: *Musa velutina*. B. *Cephaloleia dilaticollis*. RA: *Renealmia alpinia,* AP: *Alpinia purpurata,* HC: *Hedychium coronarium.* C. *Cephaloleia dorsalis*. CM: *Costus malortieanus,* CS: *Cheilocostus speciosus* D. *Cephaloleia placida*. RA: *Renealmia alpinia,* AP: *Alpinia purpurata,* HC: *Hedychium coronarium.* Letters on the bars group similar categories ($P < 0.05$).

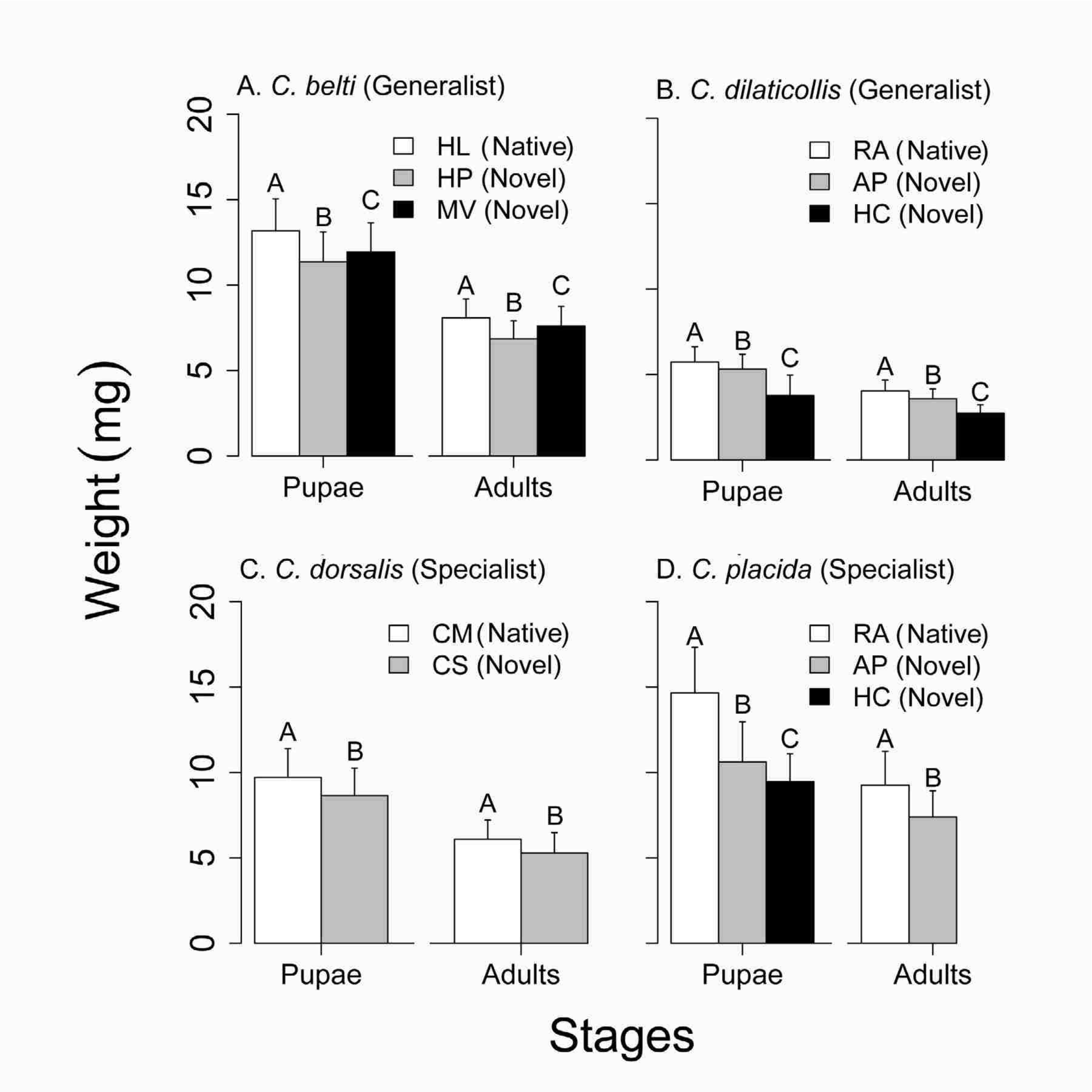

Figure 7.2. Weight (Mean ± SD) of pupae and adults of generalist and specialist *Cephaloleia* beetles reared in native and novel host plants. A. *Cephaloleia belti*. B. *Cephaloleia dilaticollis*. C. *Cephaloleia dorsalis*. D. *Cephaloleia placida*. Letters on the bars group similar categories ($P < 0.05$). Host plant abbreviations as in Figure 7.1.

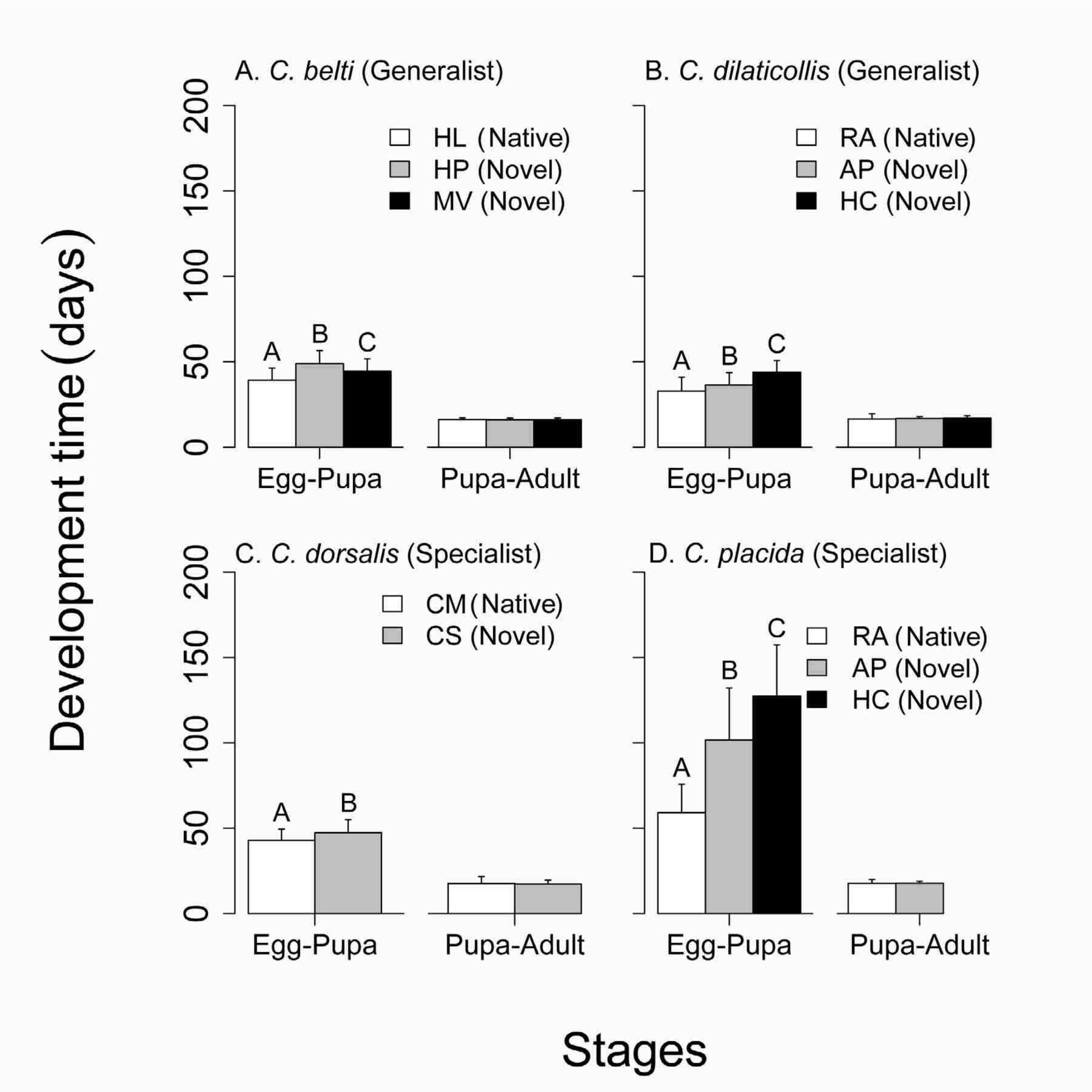

Figure 7.3. Larval and pupal development times (Mean ± SD) of generalist and specialist *Cephaloleia* beetles reared in original and novel host plants. A. *Cephaloleia belti*. B. *Cephaloleia dilaticollis*. C. *Cephaloleia dorsalis*. D. *Cephaloleia placida*. Letters on the bars group similar categories (P < 0.05). Host plant abbreviations as in Figure 7.1.

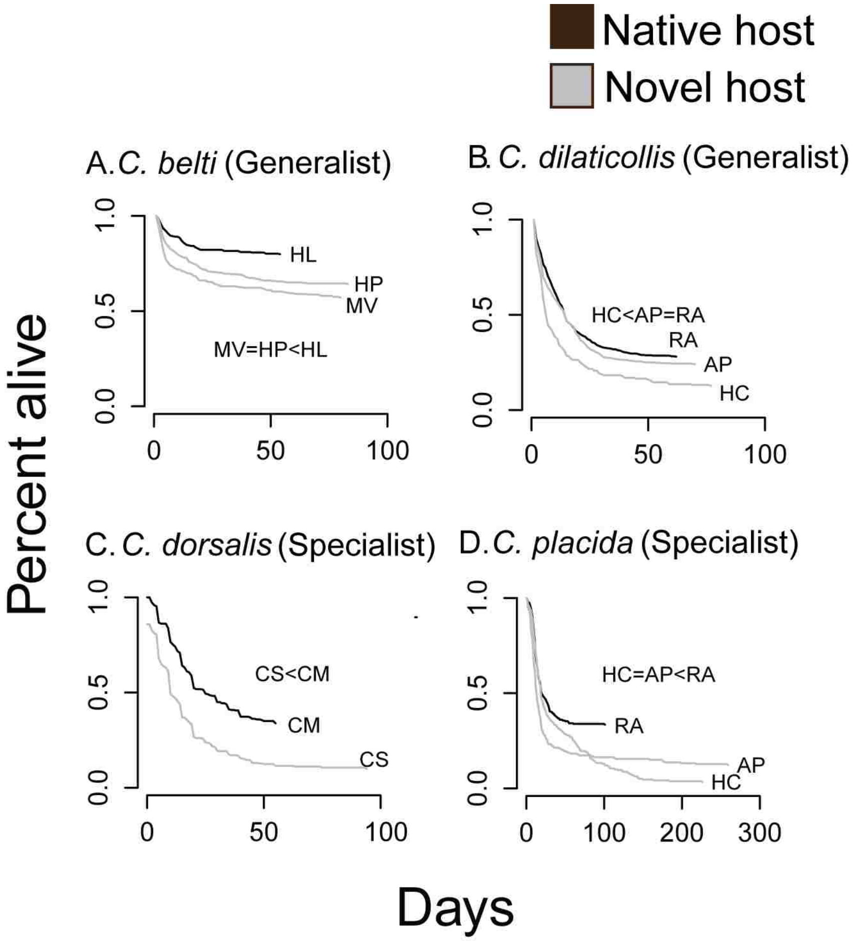

Figure 7.4. Larval survival of generalist and specialist *Cephaloleia* beetles reared in original and novel host plants. A. *Cephaloleia belti*. B. *Cephaloleia dilaticollis*. C. *Cephaloleia dorsalis*. D. *Cephaloleia placida*. Differences in survival among host plants, $P < 0.05$. Differences between host plants: Kaplan-Meier Survival Analysis with Log-rank Significance Test, $P<0.05$. Host plant abbreviations as in Figure 7.1.

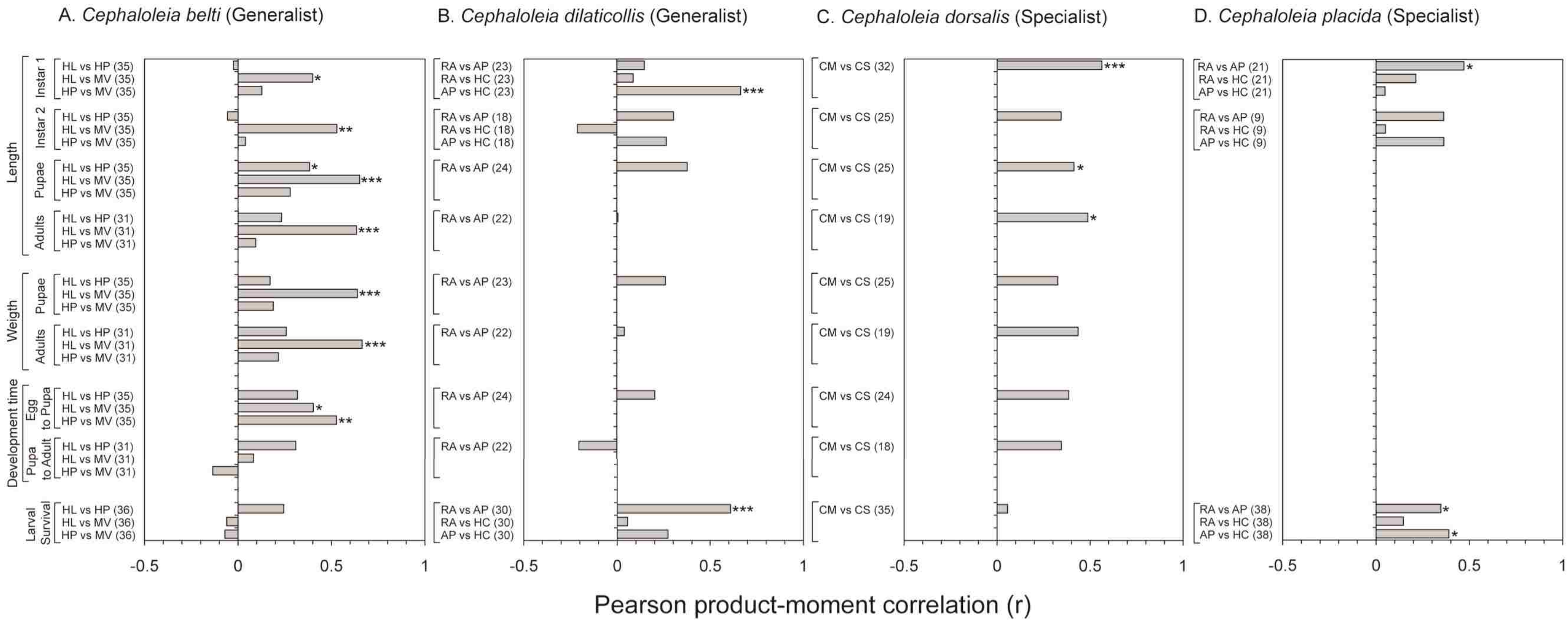

Figure 7.5. Summary of pairwise correlations for developmental traits and larval survival of *Cephaloleia* families reared in original and novel host plants. For each comparison, abbreviations indicate a host plants (Host plant abbreviations as in Figure 7.1). Sample size (number of families) is given in parentheses for each correlation. A. *Cephaloleia belti.* B. *Cephaloleia dilaticollis.* C. *Cephaloleia dorsalis.* D. *Cephaloleia placida.* Asterisks indicate levels of significance (based on Pearson's product moment correlation coefficients) *P < 0.10, **P < 0.05, ***P < 0.01.

Chapter VIII

Conclusions: adaptation, preadaptation, positive genetic correlations and the colonization of novel host plants by *Cephaloleia* beetles

In this dissertation I explored two main components of diet expansions to novel host plants by insect herbivores: the demographic and genetic responses of generalist and specialist insect herbivores feeding on novel diets. The results described within this dissertation suggest similar responses of generalist and specialist *Cephaloleia* during early colonization of novel hosts.

In *Cephaloleia* beetles, larval survival is reduced and adult longevity is increased on novel hosts. The conclusion is that both larval adaptation and adult ecological fitting play fundamental roles in diet expansions of *Cephaloleia* beetles, generating parent-offspring conflicts.

Cohort life-table analysis suggested that life history traits of both generalist and specialist herbivores display similar responses to novel environments. Estimates of instantaneous population growth rates showed that in some cases, life-history traits of populations promote the rapid incorporation of novel host plants into herbivores diets.

Quantitative genetic experiments and estimates of broad sense heritability for developmental traits and survival showed the same trend for generalist and specialist *Cephaloleia* species: positive genetic correlations in performance.

Combining the evidence from demographic and quantative genetic experiments, my overall conclusion is that the interactions between *Cephaloleia* and Zingiberales are labile. Diet expansions to novel hosts may occur without substantial evolutionary change, at least to novel host plants from the same plant family of the original host. The combined effects of adaptation, ecological fitting and positive cross-host correlations in performance described in this dissertation represent ecological and evolutionary dynamics opposite to those predicted by trade-off models. Most of the diet expantions of *Cephaloleia* to exotic Zingiberales are promoting diet generalization.

Appendix I. *Cephaloleia* beetles (Chrysomelidae: Cassidinae) at the National Museum of Natural History, Smithsonian Institution

Cephaloleia beetles (Chrysomelidae: Cassidinae)
at the National Museum of Natural History, Smithsonian Institution

Pictures: Charles L. Staines[1] Edition: Charles L. Staines[1], Carlos Garcia-Robledo[2]
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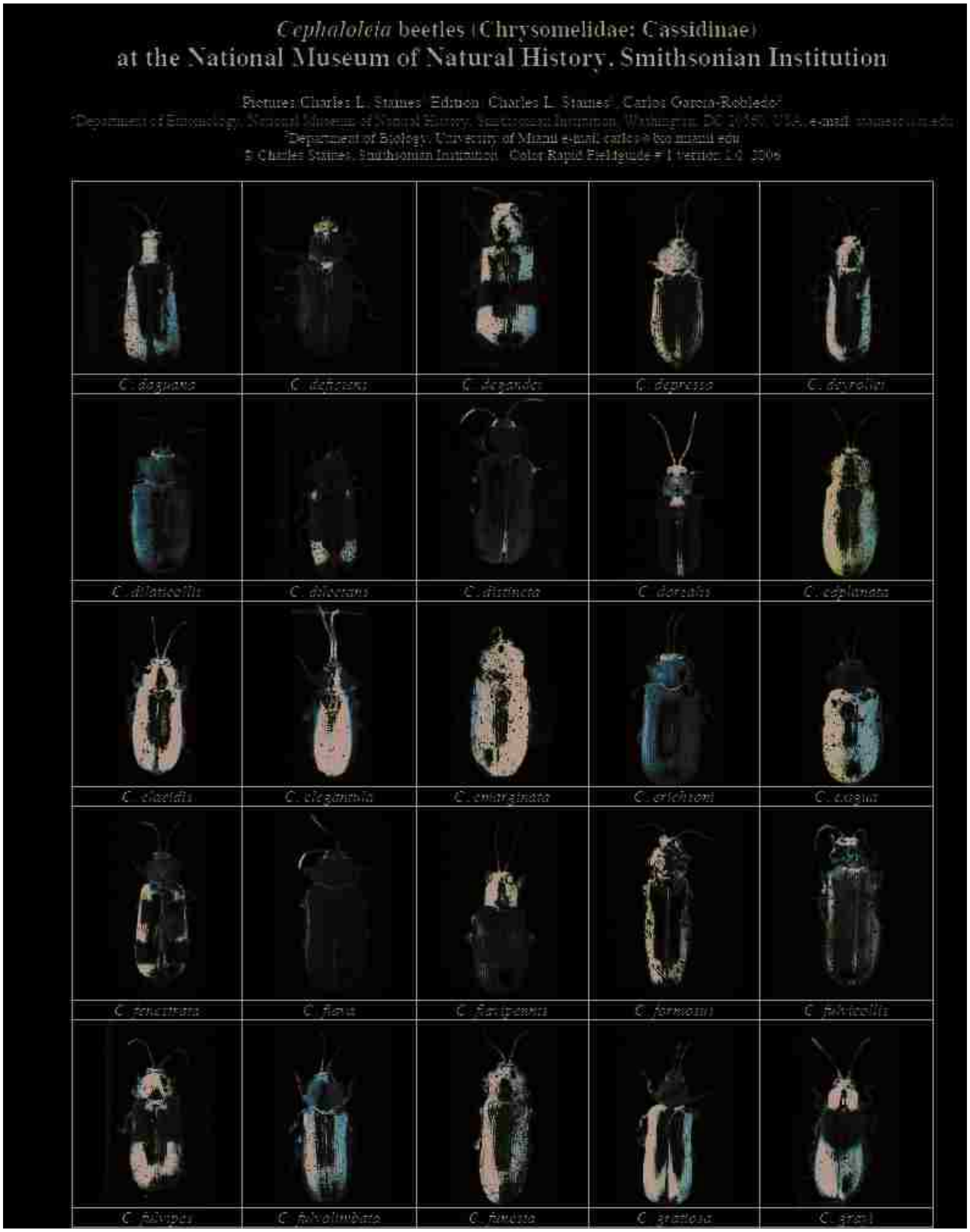

Cephaloleia beetles (Chrysomelidae: Cassidinae)
at the National Museum of Natural History, Smithsonian Institution

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Cephaloleia beetles (Chrysomelidae: Cassidinae)
at the National Museum of Natural History, Smithsonian Institution

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Cephaloleia beetles (Chrysomelidae: Cassidinae)
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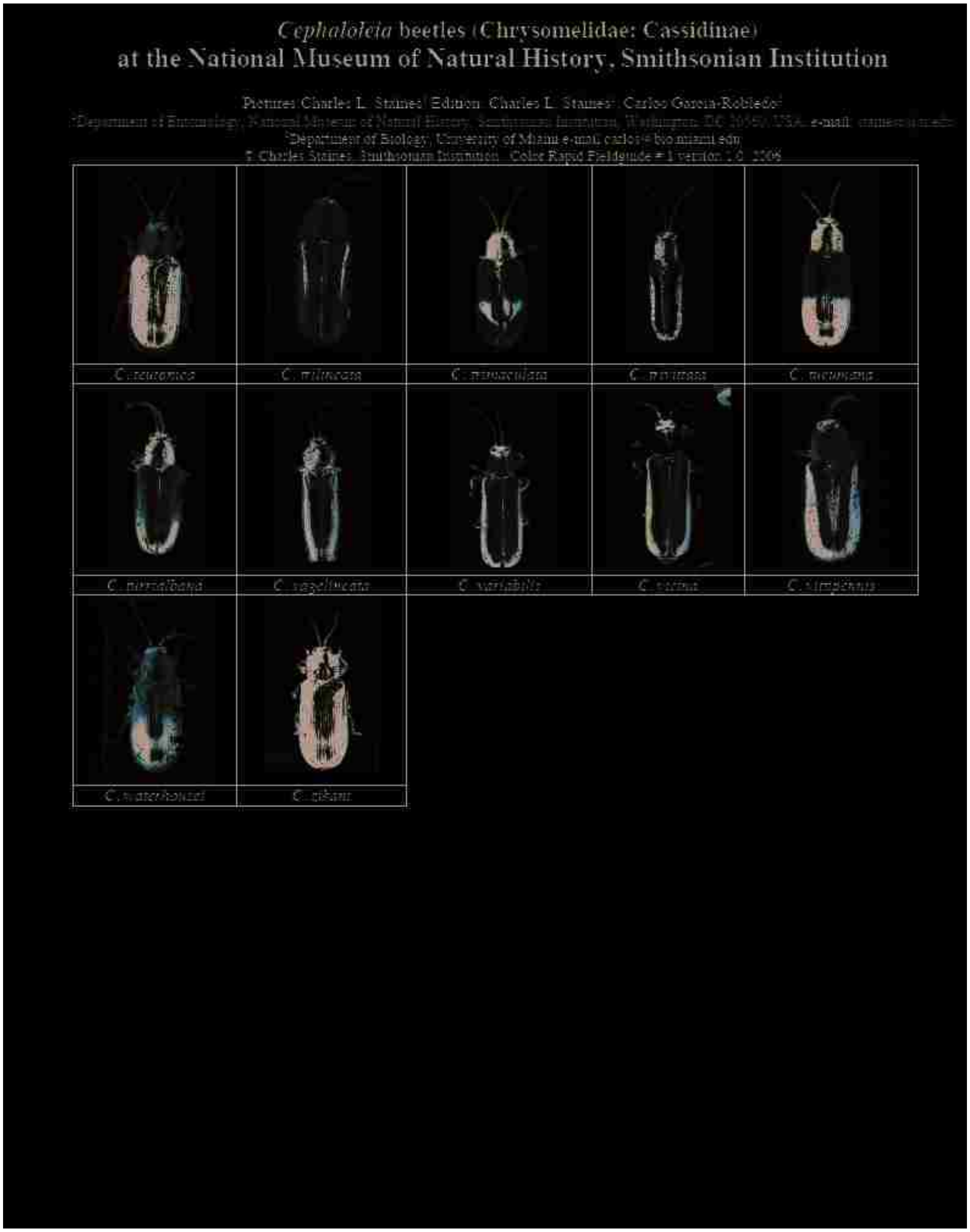

Cephaloleia beetles (Chrysomelidae: Cassidinae)
at the National Museum of Natural History, Smithsonian Institution

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C. teutonica
C. trilineata
C. trimaculata
C. trivittata
C. tucumana
C. turrialbana
C. vagelineata
C. variabilis
C. vicina
C. vittipennis
C. waterhousei
C. zikani

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