Experimental assemblage of novel plant–herbivore interactions: ecological host shifts after 40 million years of isolation

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ABSTRACT

Geographic isolation is the first step in insect herbivore diet specialization. Such specialization is postulated to increase insect fitness, but may simultaneously reduce insect ability to colonize novel hosts. During the Paleocene-Eocene, plants from the order Zingiberales became isolated either in the Paleotropics or in the Neotropics. During the Cretaceous, rolled-leaf beetles diversified in the Neotropics concurrently with Neotropical Zingiberales. Using a community of Costa Rican rolled-leaf beetles and their Zingiberales host plants as study system, we explored if previous geographic isolation precludes insects to expand their diets to exotic hosts. We recorded interactions between rolled-leaf beetles and native Zingiberales by combining DNA barcodes and field records for 7450 beetles feeding on 3202 host plants. To determine phylogenetic patterns of diet expansions, we established 20 experimental plots in the field, in which we planted plots five exotic Zingiberales, recording beetles feeding on these exotic hosts. In the laboratory, using both native and exotic host plants, we reared a subset of insect species that had expanded their diets to the exotic plants. The original plant–herbivore community comprised 24 beetle species feeding on 35 native hosts, representing 103 plant–herbivore interactions. After exotic host plant introduction, 20 percent of the beetle species expanded their diets to exotic Zingiberales. Insects only established on exotic hosts that belong to the same plant family as their native hosts. Laboratory experiments show that beetles are able to complete development on these novel hosts. In conclusion, rolled-leaf beetles are preadapted to expand their diets to novel host plants even after millions of years of geographic isolation.

Key words: Cephaloleia; Costa Rica; diet expansions; DNA barcoding; ecological fitting; herbivory; La Selva Biological Station; phylogenetic constraints.

IN PHYTOPHAGOUS INSECTS, DIET EXPANSIONS TO NOVEL HOSTS ARE USUALLY PHYLOGENETICALLY CONSERVATIVE (Goßner et al. 2009). This tendency of insects to expand their diets to closely related hosts is a byproduct of resource tracking, co-speciation, and in some cases, coevolution (Ehrlich & Raven 1964, Janzen 1980, Futuyma & Moreno 1988). After insects evolved metabolic adaptations to the unique set of chemical defenses of a particular group of host plants, phylogenetic inertia is expected to reduce insect ability to expand their diets to host plants from distant clades, which usually display different secondary compounds (Nosil 2002).

During the Paleocene-Eocene (~65 − 35 MY), many tropical plant clades became isolated in landmasses that today we know as the Paleotropics and the Neotropics (Raven & Axelrod 1974). In geographic isolation, host plants together with their insect herbivores started independent adaptive radiations (Pellmyr et al. 1998, Percy et al. 2004, Heikkilä et al. 2012).

At present, human-driven introductions of exotic plants and insects are generating the breakdown of geographic barriers

between Paleotropic and Neotropical lineages (Verhoeven et al. 2009). These encounters between previously isolated biotas represent a unique opportunity to explore the role of phylogenetic conservatism, adaptation, and preadaptation on the assemblage of novel plant–herbivore interactions (Strauss et al. 2006).

One group of plants of Cretaceous origin is the order Zingiberales (124 ± 3 MY) (Kress 1990, Kress et al. 2001). This order includes plants of economic importance such as banana (family Musaceae), ginger (family Zingiberaceae), and many ornamental plants in the families Cannaceae, Costaceae, Heliconiaceae, Marantaceae, Lowiaceae, and Strelitziaceae (Kress 1990). Most Zingiberales families originated between 110 and 95 MY ago (Kress et al. 2001, Kress & Specht 2006).

After the Gondwana vicariance and several long-distance colonization events, members of the families Costaceae, Marantaceae, Strelitziaceae, and Zingiberaceae evolved while isolated both in the Paleotropics and Neotropics (Kress et al. 2001). The family Musaceae radiated in isolation in the Paleotropics. In the Neotropics, the families Cannaceae and Heliconiaceae diversified ca. 30 MY ago (Kress & Specht 2006).

In the Neotropics, a group of insect herbivores, Cephaloleia and Chelobasis beetles (Coleoptera: Chrysomelidae) started an

adaptive radiation with their hosts, Neotropical plants in the Zingiberales (Wilf *et al.* 2000). *Cephaloleia* and *Chelobasis* beetles are also known as the rolled-leaf beetles because their life cycle is completed inside the scroll formed by the young rolled leaves of their host plants (Staines & Garcia-Robledo 2014). At present, 97 of the 214 known species of *Cephaloleia* species have been recorded feeding on Neotropical Cannaceae, Costaceae, Heliconiaceae, Marantaceae, and Zingiberaceae (Staines & Garcia-Robledo 2014). Feeding records for *Chelobasis* only include plants in the family Heliconiaceae (Staines & Garcia-Robledo 2014).

The central objective of the study described in this article was to determine whether Neotropical rolled-leaf beetles can successfully utilize exotic Zingiberales as hosts. We selected a speciose community of rolled-leaf beetles at La Selva Biological Station (Costa Rica, Central America) as a study system. We first determined the taxonomy and original (native) diets of all the insect species in this group at this site. To delimit insect species, we combined traditional taxonomy with DNA barcoding methods and recorded the associations between rolled-leaf beetles and host plants during 2 yr.

To determine if rolled-leaf beetles are able to recognize and complete their life cycle on novel host plants, we introduced four Asian and one South American exotic species of Zingiberales to La Selva and observed the occurrence of rolled-leaf beetles on these potential host plants. These exotic species were already present on the Atlantic slope of Costa Rica, but are rare inside La Selva. One possible outcome was that insect adaptation to Neotropical Zingiberales during the last 40–60 MY would prevent them from recognizing Asian Zingiberales as potential hosts (Wilf *et al.* 2000, Garcia-Robledo & Staines 2008). Alternatively, some of the rolled-leaf beetle species might recognize Asian Zingiberales as potential hosts. If rolled-leaf beetles (which are only Neotropical) could complete their life cycles on Asian Zingiberales, this would constitute an example of the enormous potential for colonization and assembly of novel interactions in insect herbivores. In this context, we addressed the following questions: (1) Are diet expansions to novel hosts restricted due to phylogenetic conservatism and the lack of an adaptive radiation with plant clades from nonnative biogeographical regions? and (2) Are insect herbivores preadapted, thus able to complete their life cycle on novel hosts after 110 million years of host plant divergence?

METHODS

Study site and species.—This study was conducted at La Selva Biological Station (hereafter La Selva), a tropical rain forest in Central America between 2008 and 2015 (McDade 1994). The study site is located in the lowlands of Costa Rica and has an elevational range of 35–137 m a.s.l. and rainfall variability of 152.0 mm in March to 480.7 mm in July. At La Selva, there are 20 morphologically distinct rolled-leaf beetle species and 33 species of native Zingiberales belonging to the families Heliconiaceae, Costaceae, Zingiberaceae, Cannaceae, and Marantaceae (García-Robledo *et al.* 2013).

For field and laboratory experiments testing the potential of rolled-leaf beetles to use exotic plants as hosts, we selected five plant species. *Musa velutina* (Musaceae), native to India, *Heliconia psittacorum* (Heliconiaceae), native to the Caribbean and northern South America, *Alpinia purpurata* and *Hedychium coronarium* (Zingiberaceae), natives to the Pacific Islands and India, and *Cheilocostus speciosus* (Costaceae), native to India (García-Robledo & Horvitz 2012). All these exotic Zingiberales had previously been introduced in the Caribbean lowlands around three decades ago as ornamentals (García-Robledo & Horvitz 2012). At La Selva, a few individuals of *Ch. speciosus, H. psittacorum*, and *A. purpurata* and naturalized populations of *H. coronarium* and *M. velutina* had previously been recorded in secondary forest and removed by the La Selva exotic plant management program (García-Robledo & Horvitz 2012).

Identification of rolled-leaf beetle species using traditional taxonomy and DNA barcoding.—To identify rolled-leaf beetle species present at La Selva, we collected 896 individuals. Beetles were independently identified by two taxonomists (C.L.S. and C.G.R) using morphological characters. In addition, to identify potential cryptic species, we amplified the DNA barcode Cytochrome C Oxidase Subunit 1 (CO1) for a subset of 397 individuals that included representatives of all morphological species collected on each host plant at La Selva.

Beetles used for molecular analyses were collected in ET-OH 95 percent. DNA extraction and amplification of CO1 sequences were performed following protocols by García-Robledo *et al.* (2013). DNA sequences were aligned using MUSCLE (Edgar 2004). We generated a neighbor-joining phylogenetic tree and estimated bootstrap support after 100 replicates using GENEIOUS PRO, v. 5.6.5 (Biomatters-development-team 2012). *Chelobasis bicolor* (Gray, 1832) and *Chelobasis perplexa* (Baly, 1858) (Chrysomelidae) were selected as the outgroup.

Determining original insect diets using field records.—To determine the diet breadths of rolled-leaf beetles at La Selva, we surveyed 3202 young rolled-leaf scrolls and recorded all the rolled-leaf beetles found in each for a total of 7450 beetles. We performed these surveys during the transition from the dry to rainy season (January–March) of the years 2008 and 2010.

Based on these records, we determined the contribution of each host plant to the overall diet breadth of each beetle species. We estimated the relative importance of each host plant in the overall diet breadth of each beetle species by calculating the average density of individuals of each herbivore species per rolled leaf for each host plant species $\bar{I}_{ij}$:

$$\bar{I}_{ij} = \frac{I_{ij}}{P_j}, \tag{1}$$

where I_{ij} is the total number of individual insects of species i found on all individuals of host plant j and P_j is the total number of rolled leaves surveyed for each host plant species j. To determine diet similarities among rolled-leaf beetles, we generated a matrix containing values of $\bar{I}_{ij}$ for each interaction, and then

performing a Bray-Curtis dissimilarity hierarchical cluster analysis (R-Development-Core-Team 2009).

Experimental diet expansions to novel host plants.—To determine if rolled-leaf beetles would find and feed upon exotic Zingiberales, we established 20 experimental plots in the field. The minimum distance among plots was 200 m. In each plot, we planted one individual of each of five exotic plant species: *Musa velutina* (Musaceae), *Heliconia psittacorum* (Heliconiaceae), *Alpinia purpurata* and *Hedychium coronarium* (Zingiberaceae), and *Cheilocostus speciosus* (Costaceae). Plants were placed in individual 5-gallon pots, each pot placed at a minimum distance of 2 m from any other. Each plant was visited every week, and reproductive structures were removed to prevent escape of genes or propagules into the wild. We recorded the presence of rolled-leaf beetles feeding in the new leaf scrolls of the plants. Beetles were collected in ET-OH 95 percent for further identification. Plants were surveyed for 3 mo, after which, all exotic plants in the experimental plots were removed from the field.

We performed two statistical analyses to determine if rolled-leaf beetles that feed on hosts from multiple plant families or insects with more host plant species included in their diets are more likely to expand their diets to exotic hosts. To determine if there is an association between the number of host plant families consumed by each insect species and the probability of diet expansions to exotic hosts, we performed a contingency analysis (Zar 1999). To explore a relationship between the number of host plant species and diet expansions to novel hosts, we performed a logistic regression between the number of host species consumed and the presence/absence of each insect species on exotic hosts (Zar 1999).

Phylogenetic constraints in rolled-leaf beetle diet expansions to novel hosts.—To determine if diet expansion onto novel hosts is phylogenetically constrained, we generated a chronogram of the tempo and mode of evolution of the main clades of Zingiberales. Time of divergence between clades is based on fossil records, molecular data, and geological information (Kress 1984, 1990, Kress *et al.* 2001, Kress & Specht 2006, Prince & Kress 2006). Using the chronogram including both Paleotropic and Neotropical clades, we estimated the time since divergence of the native and exotic plant species.

Development time and survival of rolled-leaf beetles on native and novel host plants.—To determine if rolled-leaf beetle species can complete their life cycle on novel host plants, we selected *Cephaloleia belti, C. dorsalis* (hap 1)*, C. placida, Cephaloleia* sp. nov. (see Fig. 3). We collected gravid females in the field ($N_{C.\ belti}$ = 38, $N_{C.\ dorsalis\ hap1}$ = 37, $N_{C.\ placida}$ = 42, $N_{Cephaloleia\ sp.\ nov.}$ = 32). Females were fed *ad libitum* with leaf tissue from their most frequently used host plant (Figs. 2 and 3). Eggs were placed in individual containers, and after eclosion, larvae were fed with leaf tissue from either native or the novel host plants.

For each species, we estimated the time to pupation when reared on each host plant species. We also recorded the number of individuals that completed their life cycle and emerged as adults on each host plant species. Differences in development time among insects reared on native or novel hosts were tested using nonparametric Kruskal–Wallis and Wilcoxon tests. Differences in the proportion of adults emerging from each host plant species were determined using chi-square and *a posteriori* tests (Package fifer in the statistical program/software R). All analyses were performed using program R (R-Development-Core-Team 2009).

RESULTS

Identification of rolled-leaf beetle species using traditional taxonomy and DNA barcoding.—We found 20 morphologically distinct rolled-leaf beetle species and four cryptic species complexes revealed by DNA barcoding (Fig. 1). The cryptic species included species with specialized diets that feed on a single host plant (*e.g.*, *Ch. perplexa* haplotype 1, *Cephaloleia* sp. nov), generalists that feed on several plant families (*e.g.*, *C. dilaticollis*), and even species that feed on other plant orders (*e.g.*, the *C. trivittata* complex; Fig. 2).

Chelobasis perplexa and *Cephaloleia trivittata* represent two haplotypes (Fig. 1). We also recorded a cryptic species complex for *Cephaloleia dorsalis* that is represented by three different haplotypes (Fig. 1). We found no evidence of cryptic species complexes for other rolled-leaf beetle species, including the most generalist species, *Cephaloleia belti* and *C. reventazonica* (Fig. 1).

Determining original insect diets of rolled-leaf beetles.— Most of the beetle species were specialists, feeding on a single plant family (19 beetle species) or even a single plant species (seven beetle species; Fig. 2). Even the most generalist species fed predominantly on one or a few host plants (Fig. 2).

Most beetles specialized on Heliconiaceae (Fig. 2, node 1), Marantaceae (Fig. 2, node 2), Costaceae (Fig. 2, node 3), or Zingiberaceae (Fig. 2, node 4). Five beetle species fed on multiple host plant families. We also recorded two beetle species feeding on non-Zingiberaceous species in the families Poaceae and Cyclanthaceae (Fig. 2).

Phylogenetic constraints in rolled-leaf beetle diet expansions to novel hosts.—In the experimental plots, we observed that seven rolled-leaf beetle species had expanded their diets to include exotic hosts (Table S1). Diet expansions of all rolled-leaf beetle species were phylogenetically conservative (Fig. 3). We did not generally find that insect species expanded their diets beyond the family of their native host plants (Fig. 3). The exceptions were three species of *Cephaloleia* (*C. belti, C. congener*, and *C. reventazonica*) that expanded their diets from Heliconiaceae to *Musa* in the Musaceae, a neighboring family.

There is no association between the number of host plant families consumed by rolled-leaf beetles and the probability of diet expansion to novel hosts ($\chi^2_{df = 2,\ N = 24}$ = 2.07, P = 0.35). Also, there is no association between number of host plant species and diet expansions to novel hosts (R^2 = 0.07357, df = 1,20, P = 0.51).
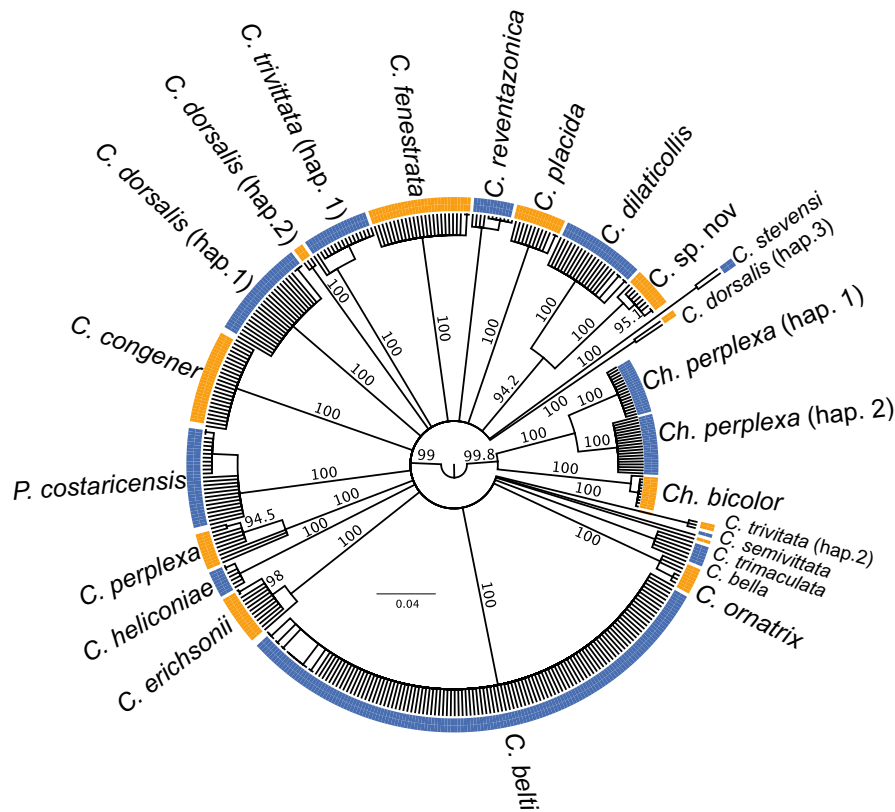
FIGURE 1. Identification of rolled-leaf beetle species at La Selva Biological Station using the DNA barcode CO1. Haplotypes (hap.) represent cryptic species based on sequence differences in the DNA barcode CO1. (GenBank submission: dx.doi.org/10.5883/DS-BOFCR, accession nos. KU357054–KU358485).

DEVELOPMENT TIME AND SURVIVAL OF ROLLED-LEAF BEETLES ON NATIVE AND NOVEL HOST PLANTS.—Larvae reared on native hosts displayed shorter time to pupation than larvae reared on novel hosts (Fig. 4). Time to pupation for *Cephaloleia belti*, *Cephaloleia* sp. Nov, and *C. dorsalis* on novel hosts was 1–2 weeks longer than on native hosts. Time to pupation in *C. placida* was more than three times longer for larvae reared on novel hosts than on their native hosts (Fig. 4).

The proportion of individuals that completed their development to adults was higher for larvae reared on native than on novel hosts (Fig. 5). Although development on some host plants was particularly challenging, and mortality was higher than 90 percent (*e.g.*, *Cephaloleia* sp. nov and *C. placida* larvae reared on *Hedychium coronarium*; Fig. 5), we recorded some individuals of each rolled-leaf beetle species that completed their development when feeding on novel hosts (Fig. 5).

DISCUSSION

A central challenge to studying plant–herbivore interaction is the limited taxonomic information available to identify interacting species (Cardoso *et al.* 2011). Previous studies using DNA barcoding demonstrated that this taxonomic impediment is a serious issue in the tropics, as many morphological species might include cryptic species complexes (Hebert *et al.* 2004, Kress *et al.* 2015).

Using the DNA barcode CO1, we recorded four cryptic species complexes at La Selva that included nine beetle species with contrasting diet breadths. This study demonstrates that if species identifications were not complemented with molecular tools, our results showing strong phylogenetic conservatism in diet expansions would have been obscured in a limited knowledge of rolled-leaf beetle species boundaries. For example, *Cephaloleia* sp. nov, *C. dorsalis*, *C. belti*, *C. congener,* and *C. reventazonica*, species recorded expanding their diets to novel hosts are included in cryptic species complexes previously assumed to display broader diets and elevational distributions (García-Robledo *et al.* 2016).

In this study, we evaluated the potential for shifts to novel hosts in a group of tropical insect herbivores with well-known phylogenetic and biogeographic history (Kress 1990, Wilf *et al.* 2000, Kress *et al.* 2001, McKenna & Farrell 2005). One hypothesis we explored was that in adapting to Neotropical Zingiberales, rolled-leaf beetles (all of which are exclusively Neotropical) may have become specialized to the extent that they would not recognize Paleotropic Zingiberales. Our data from a field experiment did not support this hypothesis; they showed that seven native *Cephaloleia* species were attracted to rolled leaves of one Neotropical and five Paleotropic exotic Zingiberales within 3 mo of their being introduced into the wild.

Host shifts are broadly documented in plant crops, such as rice and cacao, and in invasive plants (Strong 1974, 1979). Novel
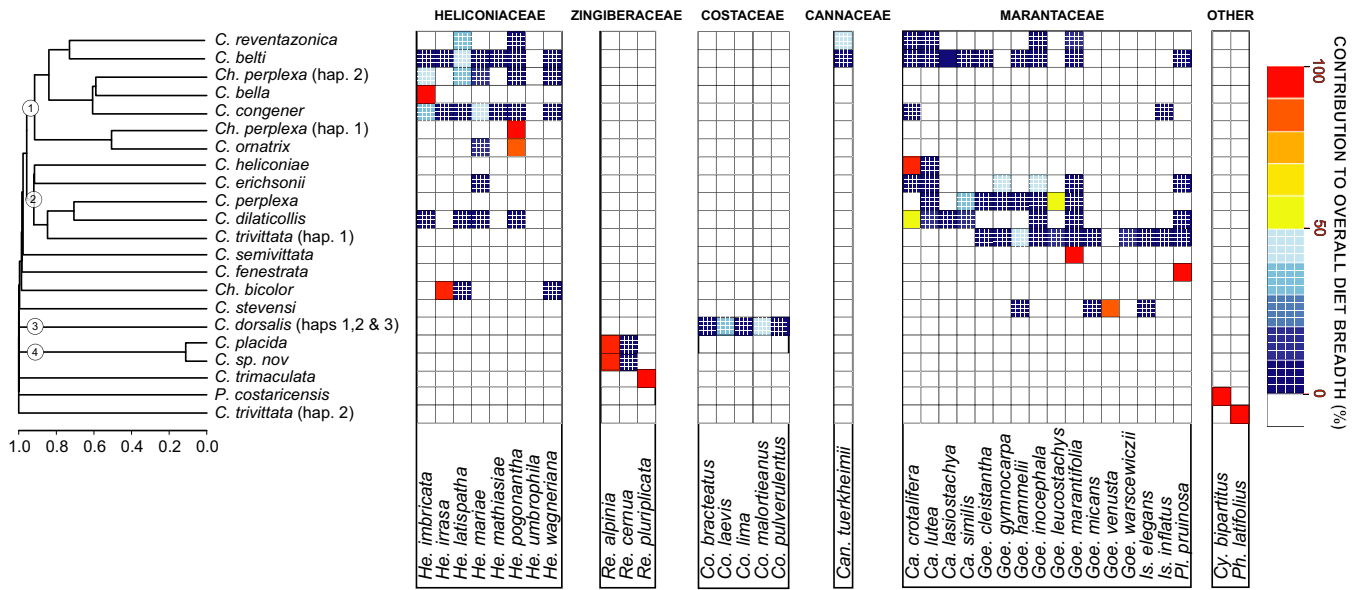
FIGURE 2. Diet similarities among rolled-leaf beetles at La Selva Biological Station. The matrix represents the relative contribution of each host plant to the overall diet breadth of each species of herbivorous insect (equation 1). Numbers on the nodes are used to describe in the text patterns of host plant use of herbivorous insects. Species of herbivorous insects are grouped using a Bray-Curtis dissimilarity hierarchical cluster analysis.
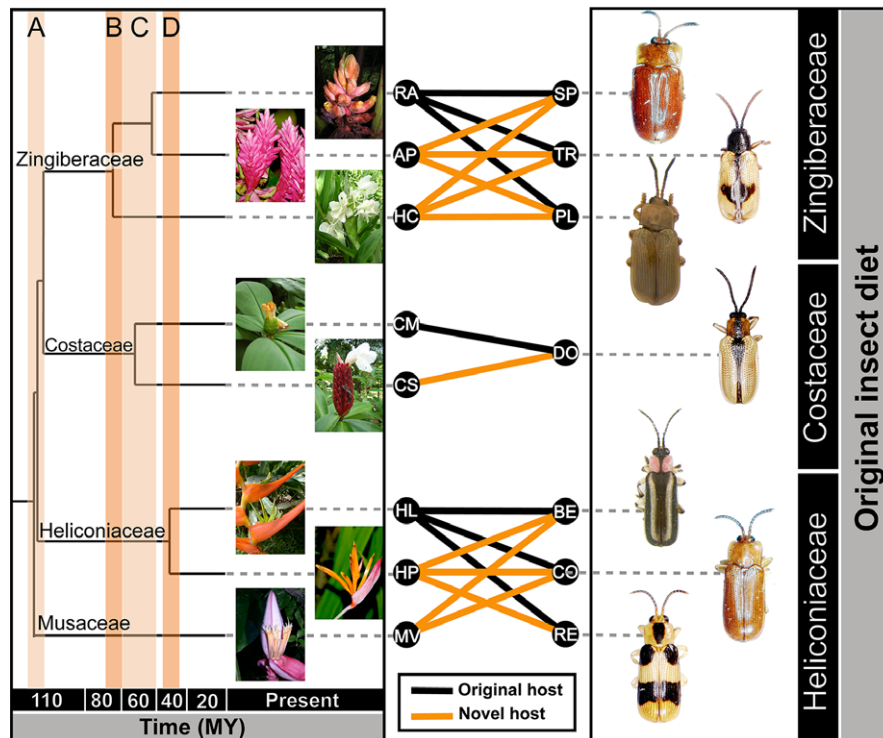

FIGURE 3. Diet expansions of rolled-leaf beetles and time of divergence between their native and novel host plants. Chronogram shows time of origin of rolled-leaf and main diversification events of their Zingiberales host plants: A. Origin of Musaceae. B. Diversification of Zingiberaceae. C. Origin of *Cephaloleia* rolled-leaf beetles and radiation of Neotropical and Paleotropic Costaceae. D. Diversification of Heliconiaceae. Bipartite network represents interactions among rolled-leaf beetles and their main native and novel host plants. Species abbreviations: RA: *Renealmia alpinia,* AP: Alpinia, HC: *Hedychium coronarium* (Zingiberaceae). CM: *Costus malortieanus,* CS: *Cheilocostus speciosus* (Costaceae), HL: *Heliconia latispatha,* HP: *Heliconia psittacorum* (Heliconiaceae), *Musa velutina,* (Musaceae). SP: *Cephaloleia* sp. Nov. TR: *C. trimaculata,* PL: *C. placida,* DO: *C. dorsalis* hap 1. BE: *C. belti,* CO: *C. congener,* RE: *C. reventazonica.*
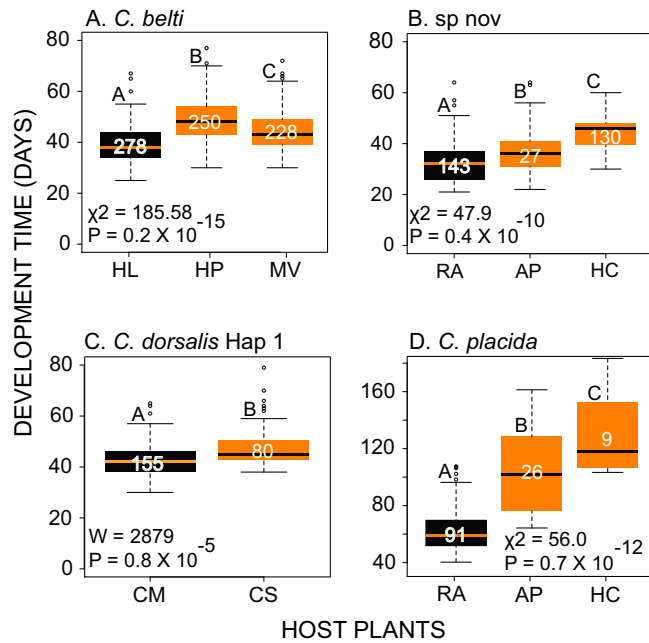
FIGURE 4. Development time from eclosion to pupation of four rolled-leaf beetle species reared on native and novel hosts (median ± 3rd quartile, 95% CI). A. *Cephaloleia belti*, B. *Cephaloleia* sp. nov., C. *Cephaloleia dorsalis* haplotype 1, D. *Cephaloleia placida*.
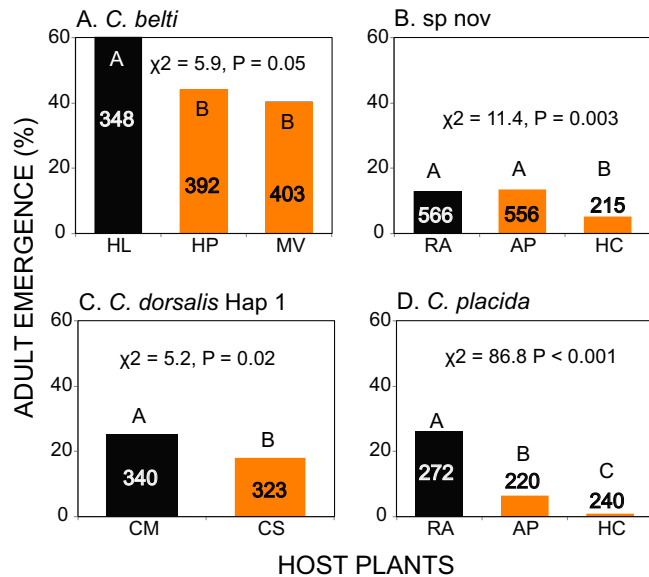

FIGURE 5. Proportion of *Cephaloleia* larvae that completed their development to adults when feeding on native or novel host plants. Numbers on the bars = sample size. Letters above bars represent similarity among groups. A. *Cephaloleia belti*, B. *Cephaloleia* sp. nov., C. *Cephaloleia dorsalis* haplotype 1, D. *Cephaloleia placida*.

plant–herbivore interactions are usually phylogenetically conservative because closely related plants display similar leaf chemistry used as cues by insect herbivores (Becerra 1997, Bernays 1998).

However, insects may also expand their diets to hosts from distant plant clades, but with similar chemical composition (Agrawal 2007).

Plants from different Zingiberales families share leaf chemistry, including those used by rolled-leaf beetles as cues to track their host plants (Merh 1982, Garcia-Robledo & Horvitz 2009). The phylogenetic conservatism of host shifts in rolled-leaf beetles suggests that this group of insect herbivores (which is only found in the Neotropics) is using chemical cues within plant families that are ancestral and predate the divergence of plants into Neotropical versus Paleotropic clades. The vicariance event that isolated Neotropical from Paleotropic Zingiberales predates the origin of rolled-leaf beetles (Specht *et al.* 2002). Therefore, the chemistry evolved prior to rolled-leaf beetle radiation in the Neotropics and did not co-evolve with the beetles.

Our data reveal that rolled-leaf beetles are preadapted to detect, colonize, and complete their life cycles on exotic hosts that are in the same family as their native hosts (Garcia-Robledo & Horvitz 2011, García-Robledo & Horvitz 2012). This suggests that the scenario of coevolution or codiversification proposed by previous studies is unlikely (McKenna & Farrell 2005, Suchan & Alvarez 2015).

The time of diversification of rolled-leaf beetles based on molecular data suggests that rolled-leaf could diversify at least 20 MY after the main radiations of Neotropical Zingiberales (Gómez-Zurita *et al.* 2007). Thus, the novel interactions we observed were assembled through a process known as 'ecological fitting', in which organisms colonize novel environments using the suit of traits that they carried at the time they encountered a novel environment rather than by evolving new trait variants (Agosta 2006, Agosta & Klemens 2008).

Our results have broad implications regarding the interpretation of one of the central hypothesis addressing the mechanisms involved in plant–herbivore diversification—*i.e.*, the coevolution—escape and radiate hypothesis (Ehrlich & Raven 1964, Janzen 1980, Futuyma & Moreno 1988). If speciation events of Zingiberales and associated herbivores were driven by the arms race proposed by this hypothesis, one prediction would be that rolled-leaf beetle expansions to Paleotropic hosts should be unlikely. Our results illustrate how the combination of conservative plant chemistry together with the immense plasticity of insect herbivore diets facilitates diet expansions to novel hosts. This study also highlights the relevance of alternative processes such as preadaptation and resource tracking in the evolution and assembly of plant insect interactions.

Novel host plants represent challenging environments for rolled-leaf beetles in the sense that larval development time is longer and mortality is higher than on native host plants (Garcia-Robledo *et al.* 2010). This study shows that although novel interactions between rolled-leaf beetles and Zingiberales are assembled through ecological fitting, insect herbivores would likely have to adapt and evolve over generations to attain fitness on novel hosts which is equivalent to their fitness on native hosts.

Humans are modifying species composition of natural areas by introducing nonnative species (Maron & Vila 2001). As a result, the assembly of novel plant–herbivore interactions is a global phenomenon (Saul & Jeschke 2015). Recent studies show that because insect herbivore diet expansions are phylogenetically conservative, it is possible to predict novel associations based on native insect diets and evolutionary relationships among native and novel hosts (Pearse & Altermatt 2013).

In conclusion, adaptation of rolled-leaf beetles to Neotropical families of Zingiberales promotes phylogenetic conservatism and constrains diet expansions to plants in the same families. However, this specialization does not represent an evolutionary dead end. Rolled-leaf beetles are preadapted to be able to expand their diets to novel hosts that are in the same family as native hosts, even after millions of years of geographic isolation.

DATA AVAILABILITY

Data available from the Dryad Repository: http://dx.doi.org/10.5061/dryad.7nk99 (Garcia-Robledo *et al.* 2017). The DNA sequences reported in this article have been deposited in the GenBank data base, dx.doi.org/10.5883/DS-BOFCR (accession nos. KU357054–KU358485). Development times and percent of emergence estimates are part of the quantitative genetics datasets of: García-Robledo C, Horvitz CC (2011). *Journal of Evolutionary Biology* 25(1): 38–53. Dataset available in dryad: https://doi.org/10.5061/dryad.c0s0h763. Dataset 1. Matrix of quantitative interactions used to generate cluster analysis (Fig. 2); Dataset 2. Data to generate Figure 4 (*Time to pupation of rolled-leaf beetles reared on native and novel hosts*); Dataset 3. Data to generate Figure 5 (*Proportion of emergence of adult rolled-leaf beetles reared on native and novel hosts*).

ACKNOWLEDGMENTS

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

TABLE S1. *Rolled-leaf beetle species attracted to novel Zingiberales host plants at La Selva Biological Station, Costa Rica.*

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