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Are chemical barriers necessary for evolution of butterfly-plant associations?

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Summary. The association between heliconiine butterflies and Passion flower vines is composed of three or more subassociations, in which each *Heliconius* species group feeds on a different *Passiflora* subgenus. The relationships are consistent with the adaptive zone hypothesis of Ehrlich and Raven, which would suggest that (1) species of the subgenus Plectostemma proliferated as a result of chemical barriers to herbivory, which created a herbivore-free adaptive zone in which speciation and diversification took place, and (2) species of the *H. erato-charitonia* group overcame these barriers and entered a competitor-free adaptive zone, in which they proliferated and speciated with those plants as hosts. The hypothesis that plant secondary chemicals were responsible for creating such barriers to herbivory was tested using heliconiine species as bioassays, in which reduced growth rates indicated presence of chemical barriers to feeding. Contrary to expectation, plants of the subgenus Plectostemma showed little or no chemical defense against any species of heliconiine caterpillar. In contrast many plants of the "primitive" subgenus Granadilla possessed significant chemical barriers against herbivory by heliconiine larvae, excepting those species in the *H. numata-melpomene* species group. I concluded that chemical barriers to feeding were not responsible for proliferation and diversification in the subgenus Plectostemma, nor did chemicals create a competitor-free "adaptive zone" in which the *H. erato-charitonia* species-group could proliferate and speciate. Chemical barriers may have been important in the evolution of the subgenus Granadilla-heliconiine association. I suggest that plant allelochemics are only one of many possible barriers to herbivory which can help create "adaptive zones" for plants and their herbivores, and that the patterns of butterfly foodplant specialization discussed by Ehrlich and Raven (1964) are not necessarily the result of biochemical adaptation and counteradaptation.

Related species of butterflies tend to feed on related species of plants (Brues 1924; Ehrlich and Raven 1964). Ehrlich and Raven (1964) suggested that these taxonomic correlations could be explained as the outcome of a coevolutionary process in which (1) plants evolve a new chemical defense which creates an herbivore-free adaptive zone in which the plants proliferate and speciate, and (2) eventually one or more insect species evolve a counteradaptation to this defense, which opens up a competitor-free adaptive zone, in which the herbivores proliferate and speciate. In this hypothesis an "adaptive zone" is defined as a region in niche space where a taxon is so successful that it proliferates into many habitats and speciates into multiple forms. Ehrlich and Raven (1964) suggested that this coevolutionary process could be responsible for the tremendous diversification seen in modern plants and herbivorous insects. Although this "adaptive zone" theory generated great interest in coevolutionary interactions between species (Gilbert and Raven 1975; Futuyma and Slatkin 1983), in chemical ecology (Rosenthal and Janzen 1979), and in the physiology of chemical barriers to feeding (Erickson and Feeny 1974), there has been little further empirical investigation into these patterns and their causes. A notable exception is the recent study by Berenbaum (1983), which suggested (1) evolution of toxic furanocoumarins created a herbivore-free adaptive zone favoring speciation in umbelliferous plants, which (2) created a competitor-free adaptive zone in which butterflies of the family Papilionidae could speciate due to their ability to detoxify the furanocoumarins.

The examples given in Ehrlich and Raven (1964) and Berenbaum (1983) suggest that adaptations involving chemical defense/detoxication systems are the primary cause of reciprocal adaptive radiations in plants and insects. However, other work on butterfly ecology suggests that host plant relations are governed by a multitude of factors such as plant habitat, phenology, morphology, associated predators, and associated plant species (Gilbert and Singer 1975; Atsatt and O'Dowd 1976; Holdren and Ehrlich 1982). Apparently, some butterfly species have evolved feeding specificity in the absence of chemical barriers (Smiley 1978a). Thus, there is no reason to expect that plant allelochemics are the only barrier to herbivory which could cause reciprocal adaptations in plants and insects.

One of the best-known insect-plant associations is the *Heliconius-Passiflora* association. Heliconiine butterflies feed as larvae only on host plants of the family Passifloraceae and the closely related Turneraceae (Janzen 1982). Benson et al. (1976) and Brown (1981) reviewed the Heliconiini-Passifloraceae interaction, and concluded that each of the three primary species-groups of *Heliconius* tend to feed on a different subgenus of *Passiflora* host plant. Species of the *H. numata-melpomene* group feed primarily on *Passiflora* subgenus Granadilla. Both taxa are considered primitive members of their respective genera, possessing morphology and behavior which is unspecialized, and from which adaptations seen in other taxa may have derived.

Species of the *H. erato-charitonia* group feed on *Passiflora* subgenus Plectostemma. Both of these taxa possess specialized morphology and behavior. Finally, species of the *H. sara-sapho* group, which are morphologically and behaviorally specialized, feed on *Passiflora* subgenera Plectostemma and Astrophaea, the last of which is morphologically primitive. This last association is believed to represent a relatively recent adaptation by the *H. sara-sapho* group.

If the *Heliconius-Passiflora* associations originated by the "chemical barrier adaptive zone" process discussed by Ehrlich and Raven (1964) and Berenbaum (1983), then it would be predicted that the *Passiflora* subgenus Plectostemma achieved its extensive adaptive radiation by evolution of an effective chemical barrier against *Heliconius* herbivory. The coevolutionary model would also predict that the *H. erato-charitonia* species-group achieved their adaptive radiation by evolving a counteradaptation to these chemicals, enabling them to colonize the *Plectostemma*. As a result, the model predicts that the *H. numata-melpomene* species-group (and other heliconiines not in the *H. erato-charitonia* line) should be unable to feed successfully on most species of subgenus Plectostemma. In addition, if the subgenus Plectostemma derived directly from subgenus Granadilla-like ancestors, the model would predict that the *H. erato-charitonia* species might retain the ability to feed on species of subgenus Granadilla. To test these predictions, I conducted a taxonomic survey of *Heliconius* larval feeding performance.

Methods

Feeding performance was tested for ten *Heliconius* species, including six of the fourteen *numata-melpomene* species and four of the seven *H. erato-charitonia* species, feeding on 16 species of *Passiflora*, including six species of the subgenus Granadilla and ten species of the subgenus Plectostemma. Larvae of three related heliconiine genera were also tested, for comparison with *Heliconius*. These genera are considered to be "primitive", i.e. similar to the characteristics of the proposed common ancestor of Heliconiini (Brown 1981). Heliconiine species were maintained in greenhouse colonies for 0–20 generations. Colonies were obtained from field sites in North and Central America, except for *H. ethilla* obtained from Belem, Brazil. During residence in the greenhouse, no changes in caterpillar feeding behavior were observed (Smiley, personal observation). The 16 *Passiflora* species tested represent only a small fraction of diversity in the subgenera Granadilla and Plectostemma. Nevertheless, most of the *Passiflora* and *Heliconius* species employed are ecological dominants, i.e. they are abundant and widespread species in most cases. Also, in many cases they are representatives of a large series of closely-related species separated geographically (Killip 1939; Benson et al. 1976), which often share similar properties (Smiley, personal observation). *Passiflora* were grown in pots in greenhouses at the University of Texas and at the University of California at Irvine, and were obtained as cuttings or live plants from field sites in North and Central America, excepting four species indicated in Table 1.

Growth tests were conducted in lighted growth chambers maintained at 25° C and 85% relative humidity with a 14/10 light/dark cycle. *Heliconius* eggs were obtained, weighed and allowed to hatch. After hatching, the larvae were placed on test plants and checked every day. Most larvae on suitable host grew rapidly and within 7–10 days moulted to the 5th instar. The time from egg hatch to pupation or death was recorded. Insects were weighed at death or 1–2 days after pupation. Comparison with field data has shown that these procedures accurately estimate growth rates of *Heliconius* under field conditions (Smiley and Wisdom in press).

Mass-specific growth rate was employed as a bioassay

Table 1. Mean growth rate of heliconiine larve raised on *Passiflora* plants. Sample size in parentheses

	Heliconius species											
	"Primitive" Genera			*Numata-melpomene* species group						*Erato-charitonia* species group			
	Agraulis vanillae	*Dione moneta*	*Dryas julia*	*ismenius*	*hecale*[a]	*ethilla*	*cydno*[a]	*pachinus*	*melpomene*[a]	*charitonia*[a]	*erato*	*clysonimus*	*hecalesia*
P. Granadilla species													
alata[a]				0.52 (5)	0.00 (5)	–	0.53 (8)	0.47 (2)	0.00 (5)	–	0.00 (2)	–	–
laurifolia[a,b]				–	0.49 (1)	–	0.60 (3)	0.57 (2)	0.56 (1)	–	0.37 (2)	–	–
ambigua[a]				0.47 (5)	0.50 (1)	–	0.53 (3)	–	0.42 (3)	0.00 (1)	0.05 (2)	–	–
serratifolia[a]				0.40 (1)	0.00 (10)	–	0.00 (1)	–	–	–	–	–	–
oerstedii[a]			0.16 (1)	–	0.44 (1)	–	0.57 (6)	–	0.53 (13)	0.35 (2)	0.24 (2)	–	–
caerulea[a]	0.39 (1)			0.57 (1)	–	–	0.56 (5)	0.50 (1)	0.52 (4)	–	0.51 (2)	–	–
P. Plectostemma species													
coriacea[a]			0.39 (3)	–	0.53 (1)	–	0.48 (7)	–	–	–	–	–	–
suberosa[a,b]				–	0.35 (1)	0.31 (1)	–	–	–	0.50 (1)	–	–	–
auriculata[a]		0.55 (1)	0.51 (1)	–	0.58 (2)	0.46 (2)	0.56 (10)	0.54 (2)	0.50 (11)	0.49 (2)	0.52 (5)	0.51 (4)	–
helleri[a]				–	–	–	0.58 (1)	–	–	–	0.55 (1)	0.57 (2)	0.66 (1)
tuberosa[b]				–	–	–	0.57 (1)	–	–	–	–	0.60 (2)	–
lancearia			0.46 (1)	–	–	–	0.53 (2)	–	–	–	–	–	–
talamancensis				–	–	–	0.62 (1)	–	–	–	–	0.60 (1)	0.63 (1)
biflora[a]		0.64 (1)	0.56 (2)	0.50 (2)	0.53 (5)	0.49 (2)	0.55 (10)	0.57 (2)	0.50 (8)	0.68 (3)	0.57 (7)	–	0.67 (2)
costaricensis[a]			0.48 (2)	–	0.42 (1)	0.46 (1)	0.55 (2)	–	–	–	0.41 (1)	–	–
capsularis	0.55 (1)		0.55 (1)	–	–	0.57 (1)	0.53 (3)	–	–	–	–	–	–

[a] Representative species used in statistical analysis of interaction between *Helioconius* species-group and *Passiflora* subgenus (Table 3)
[b] *Passiflora* species from the West Indies or Hawaii

of chemical barriers to feeding. This measure is sensitive to most toxic effects of allelochemics, as well as to chronic (as opposed to, mildly repellent) feeding deterrents. Oviposition deterrents are not directly assayed by this method. This was not considered a disadvantage, because the models of Ehrlich and Raven (1964) and Berenbaum (1983) stress toxicity effects. Nevertheless, low growth rates may be caused by feeding deterrents rather than toxicity. As a consequence, other evidence would be necessary to demonstrate the presence of toxic chemical barriers. Additionally, I have found no evidence that *Passiflora* toxins could cause sterility or reproductive failure without reducing growth rate as assayed here (Smiley 1978 b).

Mass-specific growth rates were calculated as: $r = (\ln(WP/WE))/T$, where r equals mass specific growth rate, ln equals natural logarithm, WP equals final weight, WE equals egg weight, and T equals the number of days from egg hatch to final weighing. This formula for growth rate assumes constant exponential growth, and is independent of the size of the organisms and the length of time during which growth is measured.

The growth rate data were tested statistically by analysis of variance, using a nested, two-way design which distinguished the effects of *Heliconius* species groups from variation among species within a group. The SPSS Manova program (Hull and Nie 1981) was used to calculate the statistics. The analysis indicates the statistical significance of various factors affecting growth rate, including within and between species effects, effects between species-groups, and interaction effects. The analysis was performed on the data from 5 *Heliconius* species feeding on 12 *Passiflora* species. An examination of those species excluded from the analysis (Table 1), reveals growth rates very similar to their close relatives which were included.

After determination of statistical significance, the average growth performance of each *Heliconius* species on each of the two *Passiflora* subgenera was calculated as an average of the column means in Table 1. These means were then averaged for each *Heliconius* species-group, and the grand mean calculated. The result is an average growth rate for each *Heliconius* species-group on each *Passiflora* subgenus (Table 3). Since the calculated means are themselves calculated from means, this procedure de-emphasized those *Heliconius* × *Passiflora* interactions with a large sample size in favor of emphasizing a broad taxonomic base. The analysis is thus very conservative and a t-test may be employed to test for pairwise differences (Sokal and Rohlf 1969).

Table 2. Nested two-way analysis of variance, designed to test the interaction between *Heliconius* species-groups and *Passiflora* subgenera. Analysis of growth rates from five representative *Heliconius* species fed on 12 representative *Passiflora* species (Table 2). The interaction is highly significant ($P < 0.001$)

Source of variation	Degrees of freedom	Mean square	F
Within cells	95	0.0037	
Heliconius species (within species-group)	3	0.0143	3.9*
Passiflora species (within subgenus)	7	0.0304	8.3***
Interaction between *Heliconius* species (within species-group) and *Passiflora* species (within subgenus)	20	0.0141	3.8***
Heliconius species-group	1	0.1330	9.3 $p=0.056$
Passiflora subgenus	1	0.0947	3.1 $p=1.121$
Interaction between *Heliconius* species group and *Passiflora* subgenus	1	0.4641	33.0***
Total sample size	129		

Table 3. Mean growth rate for *Heliconius* species-groups feeding on *Passiflora* subgenera. Means calculated by averaging growth rate of each *Heliconius* species on each *Passiflora* subgenus; the sample size (in parentheses) is therefore equal to the number of *Heliconius* species tested. The species growth rates were in turn derived by averaging the columns in Table 1. This procedure effectively evens out the difference in sample size between the different species, giving approximately equal weight to each

H. numata-melpomene		
on *P. Granadilla* (host)	(5)	0.45 ± 0.09
on *P. Plectostemma* (non-host)	(6)	0.51 ± 0.04
H. erato-charitonia		
on *P. Grandilla* (non-host)	(2)	0.21 ± 0.04
on *P. Plectostemma* (host)	(4)	0.57 ± 0.06

Results and discussion

Table 1 indicates that, with some exceptions, most heliconiines tested grew successfully on most *Passiflora*. The analysis of variance in Table 2 shows that growth rates were highly replicable for a given *Heliconius* feeding on a given *Passiflora*. The mean square within cells was 0.0037, which corresponds approximately to a standard deviation in growth rate of 0.05, or 10% of the mean. The between species effects were significant for both *Heliconius* and *Passiflora* species, while the effect of *Heliconius* species-group was non-significant. The interaction between *Heliconius* species-group and *Passiflora* subgenera was highly significant ($p = 0.0001$), indicating that the species-groups differ in how they interact with each *Passiflora* subgenus. It is therefore statistically valid to examine the average growth rate of each *Heliconius* species-group feeding on each *Passiflora* subgenus (Table 3), and to draw conclusions regarding the presence or absence of chemical barriers to successful growth.

Table 1 shows that most heliconiine species grew at maximum rates on most species of subgenus Plectostemma tested, including *Heliconius* of the *numata-melpomene* group. This finding conclusively refutes the hypothesis that the subgenus Plectostemma diversified in a herbivore-free adaptive zone created by a novel chemical defense against heliconiines. Thus Plectostemma, a taxon of plants potentially subject to intense butterfly herbivory, has speciated and diversified without the benefit of an effective chemical barrier against that herbivory. *Heliconius* of the *erato-charitonia* group have proliferated and speciated using this taxon of host plants, even though competing species were not excluded via chemical barriers. Apparently (in this system) reciprocal adaptive radiations in plants and insects have occurred in the absence of chemical barriers to feeding. This provides a counter-example to the model discussed by Ehrlich and Raven (1964) and Berenbaum (1983).

Growth rates of *Heliconius erato* and *H. charitonia* were greatly reduced when larvae were raised on host plants of subgenus Granadilla as compared with plants of subgenus

Plectostemma ($r = 0.21$ and 0.57, respectively, $t_4 = 7.5$, $p < 0.01$). A high percentage of mortality occurred on these plants as well. These findings, in conjunction with the observation that *H. hecalesia* and *H. clysonimus* grew poorly on subgenus Granadilla (Smiley, pers. observation) indicate that, as a whole, the *erato-charitonia* species-group does not feed successfully on most species of subgenus Granadilla (*P. caerulea* is an exception). *Dryas julia* and *Dione moneta* do not appear to feed successfully on many species of subgenus Granadilla either, although *Agraulis vanillae* apparently can do so (Table 1; unpublished observations). I conclude that many species of subgenus Granadilla are chemically protected against many heliconiine caterpillars. Apparently, only species of the *H. numata-melpomene* group have consistently overcome this barrier, and have diversified with subgenus Granadilla as principal host. On this subgenus, these insects have relatively few heliconiine competitors (Benson et al. 1976). Thus, the subgenus Granadilla and its heliconiine associates may fit the chemical barrier hypothesis of Ehrlich and Raven (1964) and Berenbaum (1983).

Chemical barriers to feeding represent only one type of anti-herbivore defense, and these findings suggest that other factors may occasionally be more important in the evolution of plant-insect associations. In most species of subgenus Plectostemma, plants are small and short-lived, and it may be that they primarily escape herbivory by being "unapparent" to herbivores (Feeny 1976), requiring specialized searching behavior to be used efficiently. Moreover, many *Heliconius* are dependent for their egg production on consumption of pollen produced by male flowers of the robust vine *Psiguria* (Gilbert 1975), which grows in the same habitat as many robust *Passiflora*, including members of the subgenera Granadilla and Astrophaea. However, members of the subgenus Plectostemma often grow in more open, sunny habitats where *Psiguria* are uncommon, and in this way may escape herbivory by *Heliconius* dependent on *Psiguria*. By this reasoning, the adaptive zone in which subgenus Plectostemma diversified may have been in part created by habitat displacement away from *Psiguria*. *Heliconius* of the *erato-charitonia* group seem to exploit to a marked extent pollen sources other than *Psiguria*, (Boggs et al. 1981), which may enable these *Heliconius* to be successful in more open habitats. Therefore, a change in pollen feeding behavior, along with pupal mating, may represent adaptations which allowed this species-group to specialize upon the Plectostemma (Benson et al. 1976). Although speculative, this hypothesis suggests one mechanism by which plant-herbivore coevolution may take place in the absence of chemical barriers to feeding.

In terms of field host plant use, the association between subgenus Granadilla and species of the *H. numata-melpomene* group is similar to that between species of the *H. erato-charitonia* group and subgenus Plectostemma. Most species prefer host plants within the association but a few species occasionally oviposit on other *Passiflora* subgenera (Brown 1981). Both associations could be interpreted as analogous examples of the "adaptive zone hypothesis," deriving from a common ancestor, but differing in the nature of the plant-defense/herbivore counteradaptation system. These findings suggest that chemical defenses do not play a unique role in mediating plant-insect coevolution. Rather, they should be considered as one of many possible elements in the antiherbivore defenses of plants, and one of the multiple barriers which herbivores eventually overcome.

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