Ode to Ehrlich and Raven or how herbivorous insects might drive plant speciation

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Abstract. Fifty years ago, Ehrlich and Raven proposed that insect herbivores have driven much of plant speciation, particularly at tropical latitudes. There have been no explicit tests of their hypotheses. Indeed there were no proposed mechanisms either at the time or since by which herbivores might generate new plant species. Here we outline two main classes of mechanisms, prezygotic and postzygotic, with a number of scenarios in each by which herbivore-driven changes in host plant secondary chemistry might lead to new plant lineage production. The former apply mainly to a sympatric model of speciation while the latter apply to a parapatric or allopatric model. Our review suggests that the steps of each mechanism are known to occur individually in many different systems, but no scenario has been thoroughly investigated in any one system. Nevertheless, studies of *Dalechampia* and its herbivores and pollinators, and patterns of defense tradeoffs in trees on different soil types in the Peruvian Amazon provide evidence consistent with the original hypotheses of Ehrlich and Raven. For herbivores to drive sympatric speciation, our findings suggest that interactions with both their herbivores and their pollinators should be considered. In contrast, herbivores may drive speciation allopatrically without any influence by pollinators. Finally, there is evidence that these mechanisms are more likely to occur at low latitudes and thus more likely to produce new species in the tropics. The mechanisms we outline provide a predictive framework for further study of the general role that herbivores play in diversification of their host plants.

Key words: coevolution; defense evolution; herbivorous insects; latitudinal gradients; plant speciation; pollination; soils; tropical forests.

INTRODUCTION

The causes of species generation in general, and plant diversification specifically, have long been debated (Schluter 2009). Ehrlich and Raven (1964) hypothesized that herbivorous insects have been a major force in plant speciation (Box 1). Because they felt that interactions between plants and insect herbivores have resulted in reciprocal speciation, they hypothesized that this process generated a large portion of current organic diversity on Earth, as encompassed by angiosperms and their insect herbivores. There is much renewed interest in the role of

herbivores in generating plant diversity (Edgar et al. 2015), especially as it applies to the tropics (Kursar et al. 2009, Coley and Kursar 2014, Becerra 2015). However, Ehrlich and Raven (1964) provided no mechanisms by which herbivorous insects might drive plant speciation, and none have been proposed since. Without specific mechanisms, it remains unclear how herbivorous insects might be the causative agents of diversification of their host plants.

Ehrlich and Raven (1964) further hypothesized that these interactions would be more potent at tropical latitudes than in extra-tropical regions (Box 1). Because low annual winter temperatures do not represent a major source of insect mortality in tropical habitats, tropical insect populations would be generally high

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Box 1. The original hypotheses

Hypothesis 1: "The evolution of secondary plant substances and the stepwise evolutionary responses to these by phytophagous organisms have clearly been the dominant factors in the evolution of butterflies and other phytophagous groups … Furthermore, these secondary plant substances have probably been critical in the *evolution of angiosperm subgroups and perhaps of the angiosperms themselves.*"

Hypothesis 2: "The abundance of phytophagous insects in tropical regions would be expected to accentuate the pace of evolutionary interactions with plants. *These interactions may have been the major factor in promoting the species*

diversity of both plants and animals observed in the tropics today."

"Probably our most important overall conclusion is that the importance of reciprocal selective responses between ecologically closely linked organisms has been vastly underrated in considerations of the origins of organic diversity. *Indeed, the plant herbivore "interface" may be the major zone of interaction responsible for generating terrestrial organic diversity.*"

Page 606 (Ehrlich and Raven 1964) (Italics are ours).

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across the year, especially compared to populations in temperate latitudes (but see Novotny et al. 2006). Resulting herbivory would be higher, leading to a greater reduction in growth and reproduction (e.g., Marquis 1991), and thus greater evolutionary pressure on plants to evolve changes in defenses. As a result, plant and animal speciation could occur at a faster pace in the tropics. Given that the vast majority of plant species occur in tropical habitats (Hillebrand 2004), much of the Earth's plant diversity could be accounted for by insect-driven plant speciation.

Both vertebrate and invertebrate herbivores are known to affect plant fitness (Marquis 2010), to effect evolutionary change in host plant traits (Agrawal 1998), to influence plant demographic processes (Crawley 1989, Ehrlén 1995, Louda and Potvin 1995, Kelly and Dyer 2002), and to influence the local abundance of individual plant species both within (Parker and Root 1981) and across microhabitats (Louda and Rodman 1996, Harley 2003, DeWalt et al. 2004, Fine et al. 2004, Lau et al. 2008). These processes, singly or in combination, are necessary but not sufficient antecedents for herbivores to drive plant speciation. Instead, herbivore impacts on plant fitness must be linked to reproductive isolation between defended and non-defended genotypes.

Here, we propose scenarios by which these linkages might arise, with the results that herbivorous insects might actually drive plant speciation. We also review the evidence that supports such scenarios. Finally, we review evidence that these processes might be more likely to occur in tropical latitudes, especially in the wet tropics. Because Ehrlich and Raven proposed that plant-insect interactions have generated much of current terrestrial biodiversity, careful review of scenarios would seem very valuable. To identify insect herbivores as drivers of plant speciation, plant traits that reduce herbivore damage must be linked to a mechanism for reduced gene flow. We focus on secondary compounds as the relevant defense traits because these traits were the focus of Ehrlich and Raven (1964); it was their goal to explain the rich diversity of secondary compounds scattered across the angiosperm phylogeny. However, physical defense traits (e.g., inflorescence spines: Hanley et al. 2009) and traits that result in ecological escape (e.g., a change in phenology)

could also lead to evolutionary escape from their herbivores. We also focus on herbivorous insects because Ehrlich and Raven (1964) emphasized the role of insects over vertebrates as instigators of plant speciation.

First, we begin with a preamble in which we elaborate the traditional coevolutionary arms race model (Ehrlich and Raven 1964, Thompson 2005). We distinguish, however, between a pathway by which reciprocal adaptation occurs without speciation and another that leads to plant speciation. We employ the biological species concept of a species, that is, species are groups of actually or potentially interbreeding populations that are reproductively isolated from other such groups (Mayr 1942). Second, to provide mechanisms for Ehrlich and Raven's first hypothesis, we describe scenarios by which insect herbivores might drive plant speciation. Finally, we review evidence for Erhlich and Raven's second hypothesis, that these processes are stronger at tropical than extra-tropical latitudes. To further encourage research in this area, we suggest research plans and potential systems that might allow testing of each of these hypotheses.

COEVOLUTIONARY SCENARIOS

The scenario of plant-insect herbivore coevolution as first envisioned by Ehrlich and Raven is one in which herbivores feeding on plants select for individuals that have novel defensive compounds (e.g., Ehrlich and Raven 1964, Berenbaum 1983, Edgar et al. 2015), or a shuffling of defensive traits that are already present (Agrawal and Fishbein 2006). The mutation would provide full or partial protection against the herbivores, while genotypes lacking the mutation would have a full complement of insect herbivores, causing sufficient damage to reduce their fitness relative to the mutants. The mutation might then sweep through the population, decreasing the average amount of damage by the herbivorous insects, and in so doing, increasing the average fitness of those genotypes. Through this process, plants can escape some or all of their herbivores, entering a "new adaptive zone" (Thompson 2005; Fig. 1a).

Because these newly evolved plants represent an empty niche for the insect herbivore, they are available for
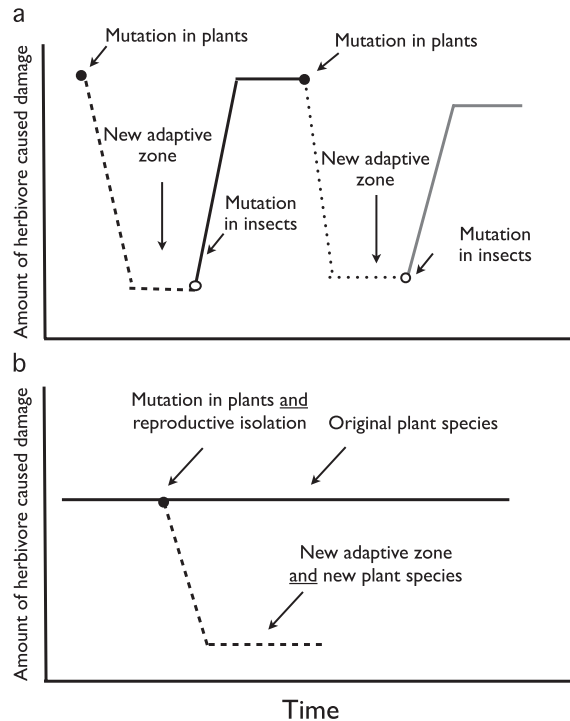
Fig. 1. Two scenarios by which herbivores may coevolve with their host plants. (a) Reciprocal Arms Race Model. Selection by herbivores favors mutations that reduce herbivory, which in turn selects for mutations in insects to use the mutant genotypes. Two rounds of adaptation and counter-adaptation are shown. Note that no speciation has occurred. (b) Speciation Arms Race Model. Selection by herbivores favors mutations that lead to reduced herbivory, and to reproductive isolation between plants of the mutant phenotype and plants of the original phenotype.

colonization by those insects, once they evolve the ability to use them. This colonization selects for further defense trait evolution in the plant, continuing the cycle of reciprocal evolutionary responses between the two parties. We call this the Reciprocal Arms Race Model (Fig. 1a). In this model, the population has a new average defense phenotype but it is ultimately the same plant species, as there has been no cladogenesis.

Ehrlich and Raven's view did not stop with reciprocal evolutionary responses between interacting gene pools, however. They proposed that patterns of host use among butterflies and their host plants give evidence that the plants are driving speciation in the insects, and in turn, the insects are driving speciation in the plants. We call this model the Speciation Arms Race Model (Fig. 1b). We feel that it is important to separate the generation of phenotypic diversity through divergent selection from the actual generation of new species. The mechanisms by which new plant species are generated by herbivorous insects have not been explored.

It is relatively easy to see how plant speciation might drive speciation in their insect herbivores (Matsubayashi et al. 2010). A new species of plant arises, slightly different in habitat preference, timing of leaf production,

and/or secondary chemistry from its sister species. Because insect herbivores often use host cues to find mates, any one of these differences would be enough to initiate reduced gene flow between the insects that use the new species of plant and the population from which they came. This is essentially the scenario for the fly *Rhagoletis pomonella* (Diptera: Tephritidae; Feder et al. 2005, Michel et al. 2007), in which new lines of *R. pomonella* have established on cherries and apples following the introduction of these plants into the range of hawthorns in North America. It is important to note that the new plant species could have originated via any speciation cause, not just herbivore pressure. However, because the new species now differs from the ancestral species in some defense trait or traits, it could cause speciation in its associated insect herbivores.

So how might insect herbivores not only cause a shift in defensive mean of a population but also cause reproductive isolation between parent and sibling populations, leading to cladogenesis (Fig. 1b)? Here, not only do herbivores select for plant mutants that escape attack by some or all members of the herbivore community, reproductive isolation arises between plants of the original phenotype and plants of the mutant phenotype. Most importantly, herbivore selection causes that reproductive isolation. We divide potential mechanisms into two major classes (Schluter 2009), those that decrease gene flow between differentially defended genotypes prior to seed production (prezygotic) and those that reduce the survival of seedlings resulting from crosses of differentially defended genotypes (postzygotic).

HYPOTHESIS 1: PREZYGOTIC MECHANISMS

A change in plant defenses could lead to reproductive isolation if there are strong relationships among defense investment, resource allocation, and reproductive traits that influence pollinator visitation. Given such relationships, any changes in plant defense traits could also alter floral traits or phenology, changing pollinator identity as a result. With a change in identity of the pollinator species, reproductive isolation may occur between the original and new genotypes, resulting in speciation (Kay and Sargent 2009, Van der Niet et al. 2014). A new plant species, characterized by a new defense regime, and associated changes in reproductive traits, would be produced sympatrically.

Links between defense and reproductive traits could influence pollinator use of a plant in two general ways (Fig. 2). Pathway 1 (dark solid arrow/red) could directly affect pollinator use if the traits that contribute to defense also contribute to pollinator attraction or repulsion. Thus a change in defensive regime could change which pollinator species visit the flowers. This would occur because the defensive compounds in vegetative tissue also serve as pollinator attractants in flowers (floral pigments: Fineblum and Rausher 1997, Armbruster 2002; floral odors: Pellmyr and Thien 1986, Coley and Kursar 2014). Second, an
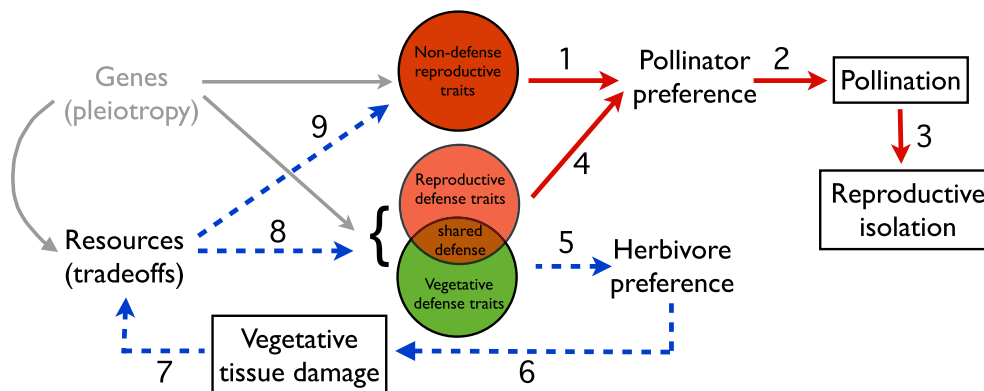
Fig. 2. Potential interactions between traits that influence defense of vegetative tissue and attract or repel pollinators. These interactions in turn may influence reproductive isolation via pollinator preference. Plant traits that are relevant fall into two general categories, reproductive traits that are not defensive in origin (e.g., flowering time, the reward constituents of pollen and nectar, inflorescence structure, anthesis, and whole plant flowering time) and plant traits that are defensive in origin. Defense traits (represented by the Venn diagram) can be further categorized into those that are uniquely found only in reproductive tissue (top circle), only vegetative tissue (bottom circle), or in both (the intersection of the two). Depending on the plant species, there could be no intersection between circles of the Venn diagram, complete intersection, or partial intersection (as shown here). By including different defensive phenotypes in a series of experiments (see Box 2), it is possible to test the role of defense on reproduction isolation through the outlined prezygotic mechanisms. All individual steps of the indicated pathways have been explored, but in no one system have all steps been completely studied. Example studies for individual pathways: (1: Raguso 2008), (2: Proctor et al. 1996), (3: Schemske and Horvitz 1984), (4: Kessler et al. 2013); (5: Berenbaum and Zangerl 1998), (6: Marquis 1984), (7: Boege 2005), (8: Adler et al. 2006, Katjiua and Ward 2006), (9: Frazee and Marquis 1994, Mothershead and Marquis 2000).

indirect effect (Pathway 2; dashed/blue pathway) could arise if changes in defense level result in reduced herbivory, which in turn provides more resources for reproductive traits. Better-defended genotypes would have more resources for reproduction, and the resultant change in reproductive traits (e.g., larger flowers, increased numbers of flowers, intensified corolla color, more allelochemicals in nectar, more nectar and pollen, and/or a shift in flowering time) would attract a different pollinator species. A potential research workflow that investigates both pathways for a single system is presented in Box 2.

Pathway 1: Coupled herbivore defense and pollinator attraction

Secondary compounds that deter herbivores could at the same time attract or deter potential pollinators (Raguso 2008). A number of volatiles have been shown to attract both herbivores and pollinators (Kessler and Halitschke 2009, Lucas-Barbosa et al. 2011). Other volatiles can serve to dissuade herbivores from eating leaves (Kessler and Halitschke 2009) or flower parts (Kessler et al. 2013). Thus a change in defense could change the attraction of particular pollinator species. In Nyctaginaceae, for example, both foliage and flowers emit the same compounds (Levin et al. 2003, see Coley and Kursar [2014] regarding *Inga* [Mimosaceae]). In a detailed study, Raguso et al. (2003) identified 125 volatiles from nine species of *Nicotiana* (Solanaceae), 28.3% (range = 5.8–92.6%) of which are produced by both flowers and leaves. Because a number of these volatiles are known to have a defensive function and because different compounds

attract different pollinator species (Raguso et al. 2003), a change in defensive compounds could change the identity of the pollinator. Finally, an evolutionary shift in leaf volatiles could potentially have a direct effect on which pollinator species visit a flower. In *Silene* (Caryophyllaceae), volatiles released following herbivore attack to leaves increase fruit set via increased pollinator attraction (Cozzolino et al. 2015).

In a similar vein, various secondary compounds, including chalcones, anthocyanins, flavonoids, and alkaloids, can serve as herbivore deterrents (Waller 1978, Appel 1993, Lattanzio et al. 2006) but also provide color to floral parts (Fineblum and Rausher 1997, Irwin et al. 2003, Whittall and Strauss 2006). Gronquist et al. (2001) demonstrate that the same compounds that underlie UV patterns in *Hypericum calycinum* also are found in pollen and the ovary wall and are toxic to at least one insect species. Differences in corolla color can influence which pollinator species visit a plant (e.g., Stanton 1987, Melendez-Ackerman and Campbell 1998), while damage to flowers can influence pollinator service (Krupnick et al. 1999). Large shifts in floral color and associated pollinator attraction are linked to plant speciation (Schemske and Bradshaw 1999, Hodges et al. 2002, Bradshaw and Schemske 2003).

Finally, compounds in vegetative tissue could protect against herbivores, but influence potential pollinators if these compounds are also found in nectar (Adler 2000) or pollen (Kessler and Halitschke 2009). For example, alkaloids deter bumble bees (Gegear et al. 2007), while caffeine enhances the memory of honey bees for rewards (Wright et al. 2013). Both alkaloids in general (Knolker

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Box 2. A workflow for testing prezygotic mechanisms by which herbivores might drive plant speciation

The best system to test the pathways in Fig. 2 would be one in which the major herbivore species, leaf and floral chemistry, and pollinator species are all known, including potential pollinators in the habitat that currently do not visit the plant but might do so if there was a shift in floral traits or flowering time.

Experiment 1: Manipulate resources independently of herbivore presence to confirm that floral trait expression is influenced by resource availability, Steps 8 and 9. Experiment 2: Manipulate resources independently of herbivore presence and pollinator visitation (using hand pollination) to confirm that seed production is resource limited, Steps 9-1-2, and Steps 8-4-2. Experiment 3: Measure the relationship between secondary chemical composition, herbivore damage, and pollinator visitation under natural conditions, Steps 5-6-7-9-1; Steps 5-6-7-8-4. Experiment 4: Use native herbivores caged on plants to cause leaf tissue loss, Step 6. Measure traits of flowers produced subsequently on heavily eaten vs. damage-free plants, including flower size and color, nectar production, nectar composition including both rewards and deterrents, pollen production, and floral odors (e.g., Frazee and Marquis 1994, Strauss et al. 1996), Steps 7-9, 7-8. Experiment 5: Expose plants

to a controlled number of relevant pollinator species (two species at a time, one of which currently is the main pollinator and the other of which only occasionally visits the flowers) under natural conditions (e.g., Galen 1989) to test for preferences between experimental and control plants, Steps 1 and 4. Data would be analyzed to determine which floral traits influence pollinator choice.

The predictions of these experiments and analyses would be: 1) resource availability influences floral traits, which in turn influences pollinator visitation (Steps 8 and 9); 2) leaf chemistry influences the amount of damage (Steps 5-6); 3) undamaged and damaged plants differ in at least one floral trait that influences which species of pollinator visit the plant (Steps 7-9 and 7-8); 4) different species of pollinators prefer control vs. experimental plants (Step 1 and 4); 5) both direct pathways (Steps 1-2, 4-2) and indirect pathways (5-6-7-9-1-2; 5-6-7-8-4-2) will contribute to seed production when defensive compounds also influence pollinator behavior via floral traits; 6) the largest shifts in the pollinator species composition after herbivory will occur for plant species in which herbivory reduces resource availability and these resources influence both defense traits that protect floral tissue and non-defense reproductive traits.

2013) and caffeine in particular (Nathanson 1984) are known to deter herbivores. Ultimately, the balance sheet for whether there are negative or positive effects of changing secondary compounds on pollinators and herbivores will depend on whether the floral compounds deter or encourage visitation by beneficial vs. detrimental floral visitors (Adler and Irwin 2005).

Perhaps the best-documented system linking the evolution of defense, pollinator attraction, and plant speciation is that of African and New World *Dalechampia* (Euphorbiaceae; Armbruster 1997). In this system, resins that protect leaves are a subset of those collected by Megachilidae bees from *Dalechampia* flowers (Armbruster et al. 1997). The bees pollinate the plants in the process of collecting the resins (Armbruster et al. 1997). Differences in resin composition and time of floral opening might influence which bee species visit which plant species (Armbruster and Herzig 1984). Armbruster and Herzig (1984) and Armbruster (1997) propose that resins originally arose as defensive compounds in bracts of staminate flowers and subsequently evolved as attractants for pollinators and defenses in leaves and shoot tips in this system. Analysis of additional taxa supports this conclusion but reveals a complex history of gain and loss of traits (Armbruster et al. 2009).

Theoretical considerations suggest there would be strong selection to decouple the compounds that attract pollinators from those that dissuade herbivores (Armbruster et al. 1997). In uncoupled systems (nonoverlapping circles in the Venn diagram, Fig. 2), evolution of defensive chemistry would not be constrained by a correlated response in compounds that attract pollinators, and vice versa. Such decoupling would reduce the likelihood of changes in defensive chemistry influencing plant-pollinator interactions (Hanley et al. 2009, Adler et al.

2012), and as a result, herbivore-driven speciation through these shared pathways of herbivore deterrence and pollinator attraction. As a counterbalance, production of secondary compounds is very phylogenetically constrained (Ehrlich and Raven 1964). Hence, many plant species may not have the biochemical flexibility to evolve different sets of defensive and attractive compounds. Theory aside, we do not know how often secondary compounds are involved in both herbivore deterrence and pollinator attraction, only that a number of examples have been demonstrated (e.g., Levin et al. 2003, Adler and Irwin 2005, Theis 2006).

Pathway 2: Coupled herbivory and resource allocation to pollinator attraction

Even if the compounds that deter herbivores differ from those that attract pollinators, shifts in foliar defense can still cause reproductive isolation to arise. A change in defense traits, leading to reduced tissue loss, can increase resources available for reproduction (Bazzaz et al. 1987). Evidence linking defense and allocation to reproduction comes from the effect of experimental defoliation and exclusion of herbivores. Leaf damage, for example, often delays flowering (e.g., by as much as a month in *Piper arieianum* (Piperaceae), Marquis 1988). The assumption here is that better defended genotypes would flower earlier, and as a result not exchange pollen with later-flowering genotypes. The shift in flowering time will only result in reproductive isolation if there is a suitable pollinator species available, either the same species or a different one. The latter is most likely to occur in strongly seasonal environments in which pollinator community composition is changing continuously. Pollinator species identity varies across the flowering season for a number

of species (e.g., Ashman and Stanton 1991, Cane and Payne 1993). The time scale could be months, days (e.g., Baldock et al. 2011), or within 24 h. For *Nicotiana attenuata*, unattacked (night-flowering) plants are pollinated by hawk moths while attacked plants flower during the day, and as a result are pollinated by hummingbirds (Kessler et al. 2010). The likelihood of herbivore attack in this system is influenced by differences in secondary chemistry (Kessler et al. 2010). Thus, a shift in defense could result in reproductive isolation in this system.

Opposing these changes in some systems would be strong selection against asynchrony either in leaf flushing or flowering. If synchronous flushing satiates a specialist herbivore species (Aide 1988), there would be selection against shifts in flowering that are genetically coupled to the time of leaf flushing. Similarly, if the pollinators are only specialists who operate over a short period of time, there would be strong selective pressure to maintain synchronized flowering (Augspurger 1980). A generalist folivore community that varies little in time or an herbivore community that is strictly controlled by the third trophic level would favor drift in flowering time (Angulo-Sandoval and Aide 2000).

Folivory can also result in changes in flower size, inflorescence display, nectar amount, nectar concentration, and pollen production (see review by Whittall and Strauss 2006). Changes in these characteristics could cause a shift in the pollinator species visiting the plant as a result of increased defense and reduced herbivory. Folivory in *Oenothera macrocarpa* (Onagraceae; Mothershead and Marquis 2000) reduces both corolla width and nectar tube length. As a result, flowers are visited less by their hawk moth pollinators, and visitation is less likely to produce seeds because hawk moths do not come in contact with floral sexual parts of experimental plants. In this scenario, if there are smaller pollinator species available, visitation could still result in adequate pollination. Small pollinator species would visit small-flowered plants, and large pollinator species could visit large-flowered plants. This association between defense type and pollinator species could result in reduced gene flow, divergence, and finally speciation.

For each of these scenarios in which defense subsequently influences resource availability through reduced herbivory, the gain in resources as a result of protection is balanced against the cost of the new or alternative defense. If the cost of defense exactly equals the benefit of protection, then there would be no net gain in resources that could lead to increased allocation to reproduction. However, there are systems in which alternative defense compounds would incur little or no additional resource investment (e.g., isomers: Berenbaum et al. 1989).

Pathway 3: Coupled defense and stigma-pollen interactions

There is some suggestion that there is "cross talk" between plant defense and pollen-stigma interactions

(Rejón et al. 2014). Stigma exudates can contain flavonoids that influence pollen germination, and at the same time these flavonoids may protect vegetative tissue against herbivores. Pollen exudates can also contain flavonoids. These pollen exudates can negatively affect pollen germination, pollen tube growth, and stigma receptivity, perhaps because of the presence of the flavonoids. Changes in defense type or allocation could therefore influence which genotypes may produce seeds, restricting gene flow.

A limited number of studies have analyzed stigma and pollen exudates. Phenolics have been found in extracts of stigmatic exudates in a phylogenetically diverse set of plant species (e.g., Martin 1969, Sedgley 1975, Gonzalez et al. 1994). Vogt et al. (1994) showed high levels of the flavonol aglycone kaempferol in stigma exudates following pollination in *Petunia hybrida* (Solanaceae), while addition of kaempferol to stigmas increases pollen germination in that same species (Pollak et al. 1993). This compound is also found in the leaves of this plant species. Twenty different allelochemicals, including flavonoids, have been described from pollen exudates (Murphy 2002). The exudates from heterospecific pollen have been shown to inhibit target pollen germination at pollen grain numbers that naturally occur on stigmas (Murphy 2000). An untested hypothesis is that conspecific pollen from plants of different vegetative defense profiles might differentially inhibit each other at the stigmatic surface, and in this way lead to reproductive isolation.

HYPOTHESIS 1: POSTZYGOTIC MECHANISMS

In this general set of mechanisms, seeds are produced as a result of matings between differentially-defended genotypes but herbivores either kill the hybrid seeds or reduce the survivorship of plants derived from those seeds. In contrast to the above mechanisms, reproductive isolation would follow as result of strong disruptive selection. This original disruptive selection, and subsequent divergence, could be driven by: (1) forces other than herbivory, with subsequent selection against hybrids in hybrid zones; (2) herbivory on plant populations occupying parapatric microhabitats; or (3) spatially variable herbivore communities on plants occurring in allopatry with subsequent selection against hybrids. It is possible that traits influencing herbivore attack via the third trophic level may be important (e.g., Léotard et al. 2008). A research workflow aimed at examining the role of postzygotic mechanisms for herbivore-driven plant speciation is outlined in Box 3 and Fig. 3.

Pathway 4: Selection against hybrids in hybrid zones

Herbivores could reinforce species boundaries under scenarios in which plant populations have diverged in allopatry, but then come together geographically. Herbivores, if they preferentially feed on hybrids, could select for reduced gene flow between the diverging

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Box 3. A workflow to test for postzygotic mechanisms by which herbivores might drive plant speciation

The best system would be one in which hybridization occurs in sympatry between closely related, but widely distributed plant species. Reciprocal transplants of hybrids and pure parental types from around the hybrid zone, coupled with insect exclosures will test for the effects of insect herbivores on plant survival and growth, as well as any abiotic effects of local environment in the absence of herbivores (Fine et al. 2004). If herbivores are reinforcing species boundaries, then herbivore abundance should be greatest on hybrids, and the resulting damage higher and fitness lower (Pathway 4). If parental types occur in different habitat types, survival and growth in the presence and absence of herbivores will reveal whether tradeoffs between defense and abiotic factors influence plant distribution (Fine et al. 2004 (Pathway 5)). Genetic tests should be conducted to confirm the genetic identity of each experimental plant. Reciprocal transplants across large distances, coupled with herbivore exclusions, could reveal how success in one part of the geographic range of a plant is due to adaptation to the local abiotic conditions vs. adaptation to the local insect community (Pathway 6). In theory, one could then identify genes associated with these apparent adaptive differences.

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populations, reinforcing speciation in the process. In the hybrid zone between *Salix eriocephala* and *S. sericea* (Salicaceae), herbivores selectively feed on hybrids (Orians et al. 1997). In the understory rainforest tree *Leonardoxa africana* (Fabaceae), there are two subspecies, *L. a. africana* and *L. a. gracicaulis*. The former has domatia and is protected by ants, and the latter does not have domatia but has synchronized leaf flush that satiates herbivores. Hybrids between the two, which occur in N'kolobonde, Cameroon, have intermediate traits, suffer higher herbivory than both parental populations and exhibit lower growth rates (Léotard et al. 2008). However, herbivores do not always selectively feed on hybrids, and in fact many times prefer one or both parental genotypes (e.g., Drew and Roderick 2005; Appendix S1). By doing so, they can serve as a pathway by which alleles conferring defense may move from one parental type to the other. Consistant with this scenario, evidence suggests that the subspecies *Helianthus annuus texanus* (Asteraceae) has arisen via adaptive introgression of alleles moving from *H. debilis* to *H. annuus annuus*. These alleles provide resistance to receptacle- and seed-feeding herbivores (Whitney et al. 2006).

Pathway 5: Defense evolution in parapatry

Anti-herbivore defense allocation among plant species can be differentially costly, especially between habitats that vary extremely in resource availability. The colonization of resource-poor environments is hypothesized to select for high defenses due to the high cost of tissue loss in those environments (Coley et al. 1985). If mutations occur that allow plants to colonize one habitat type from another, there will be selection against hybrids as a consequence of environment-related differences in defense investment. The result could be species formation driven by herbivores.

Fine et al. (2004) demonstrated that insect herbivores are responsible for habitat partitioning by various Amazonian tree species between clay and white sand soils. On white sand soils, white sand soil specialists outcompete clay soil specialists because the former are better defended than clay specialists. White sand soil specialists invest more in defense because of lower resource availability on white sands (Janzen 1974, Coley et al. 1985). As a result they cannot invade clay soils even if herbivores are absent because clay soil species invest less in defense and thus can grow faster. Incipient speciation is occurring in *Protium subserratum* (Burseraceae; Fine et al. 2013, Misiewicz and Fine 2014), in which there are three different chemical ecotypes, each found on a different soil type (Lokvam et al. 2015). Differences in soil chemistry on the three soil types are associated with differences in herbivore communities and different leaf defensive chemistry.

Tradeoffs between defense allocation and growth rate across neighboring microhabitats may enhance the selection process against hybrids. The relevant factors that determine the likelihood of this type of herbivore-driven speciation are the strength of herbivore pressure, the steepness of resource gradient, and whether tradeoffs exist for one or both microhabitat specialists. Suppose, for example, that there are no tradeoffs but the herbivore community varies by microhabitat. In this case herbivores alone could select for reduced gene flow. However, if there are additional negative tradeoffs in defense allocation and growth in one or both microhabitats, the speciation process might proceed at a faster pace. Positive tradeoffs could also occur if soil elements such as nickel act as herbivore deterrents (Boyd and Martens 1994).

Pathway 6: Geographic mosaic selection in allopatry

The hybridization scenario described in Pathway 5 could be expanded to a larger geographic context. Various populations of the same plant species are often attacked by different herbivore species (e.g., Compton et al. 1989). This geographic variation in herbivore ensembles could select for differences in defensive compounds through strong disruptive selection. This is essentially a component of the Geographic Mosaic Theory of Coevolution (Thompson 2005). Eventually, plant populations could diverge sufficiently to become reproductively isolated. The difficulty with this scenario is demonstrating that herbivores have driven speciation, and not some other factor correlated with herbivore community composition. Soil type can influence insect
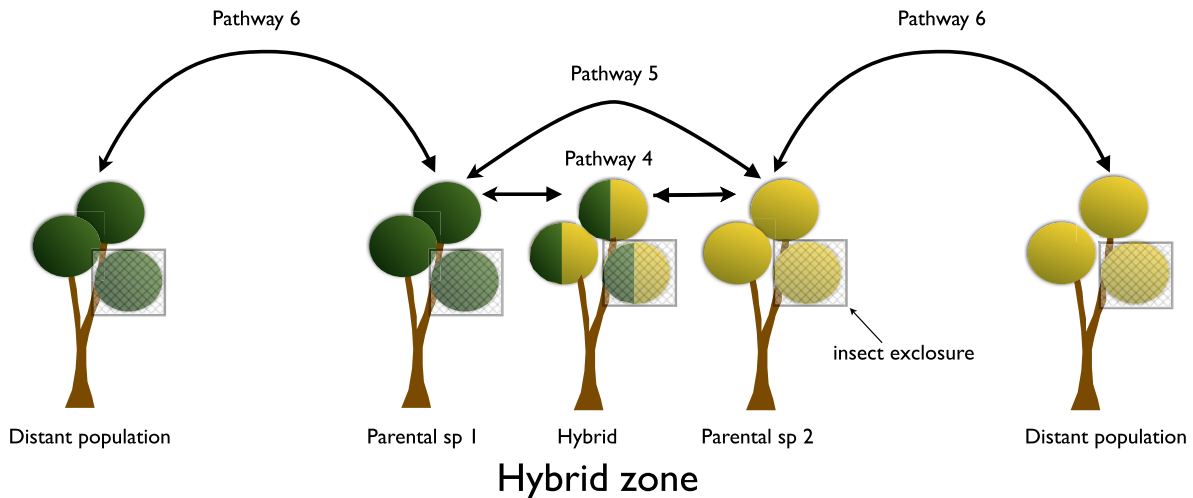
Fig. 3. Reciprocal transplant design, coupled with insect exclosures, to test for the effects of insect herbivores on differentiation and reproductive isolation of plant clades with respect to defense. The dark (green) vs. lightly (yellow) shaded trees represent different genotypes, subspecies, or closely related species. Arrows represent the source and destination of reciprocal transplants. Different parental species might occur in different microhabitats or not. Systems involving ants (e.g., Léotard et al. 2008) would need to include a third experimental factor, i.e., the presence or absence of ants. See text for discussions of pathways.

herbivore community composition (e.g., Fine et al. 2004), but soil type variation is not necessary for changes in herbivore community composition as composition varies over even short distances on the same soil type (Barber and Marquis 2011). For speciation to be driven by herbivores in this way, these spatial differences in herbivore communities would have to be consistent for at least one generation of the host plant, but it is likely that many generations are required. Janz (2011) proposed his "oscillation hypothesis," which might explain how host plant driven herbivore diversification could also drive plant speciation through interactions with herbivores, but in the absence of chemical diversification.

Hypothesis 2: Are These Processes More Likely to Occur in the Tropics?

For any of these scenarios to be more effective in the tropics, as proposed by Ehrlich and Raven, herbivore pressure must be greater at lower latitudes. Most of the currently available evidence for this latter hypothesis suggests that there is often no difference in herbivore pressure with latitude. When there is a difference, however, it is in the hypothesized direction. Coley and Barone (1996) found evidence for higher folivory at lower latitudes based on their survey of literature values, while Moles et al. (2011) measured folivory across many plant species and many latitudes on a global scale, and found no latitudinal trend in leaf damage. Poore et al. (2012) compared feeding rates on algae in a meta-analysis of herbivore exclusion studies in marine systems, finding no latitudinal trend, while Pennings and Silliman (2005) found higher feeding rates on saltmarsh grasses at low

latitude than at high latitude. Studies that control for phylogeny and measure secondary compounds in the same way are needed to conclusively test the hypothesis (Marquis et al. 2012).

For the proposed prezygotic mechanisms to be effective, there must be potential pollinators in the habitat that are not currently using the plant species, but which would be available if there were defense-related changes in pollinator attraction. Given that there are more pollinating insect and bird species at low latitudes than at high latitudes (Ollerton and Cranmer 2002), this mechanism is more likely to be effective at low latitudes. For example, there are 150 species of Sphingidae hawk moths in the Área de Conservación Guanacaste in Costa Rica (110,000 ha in area; Janzen and Hallwachs 2009), but only 44 species in the state of Missouri, USA (P. Koenig, *unpublished data*), which is 161 times larger in area. Thus a plant genotype in Costa Rica whose flower size has shifted, for example, is more likely to encounter a different hawk moth species that is capable of pollinating the new genotype than if a similar shift occurred in Missouri.

Postzygotic mechanisms will be more effective in the tropics only if the tropics offer higher beta diversity, whether of microhabitats or herbivore assemblages. A study by Myers et al. (2013) provides evidence consistent with this requirement, as they found that beta diversity in soil types is greater between tropical forest plots in Bolivia than between temperate forest plots in Missouri.

We do not know if tradeoffs in defense and growth due to limited resources are more likely at low latitudes. This might be true if defenses are costly, for which there is equivocal evidence depending on the system (Koricheva
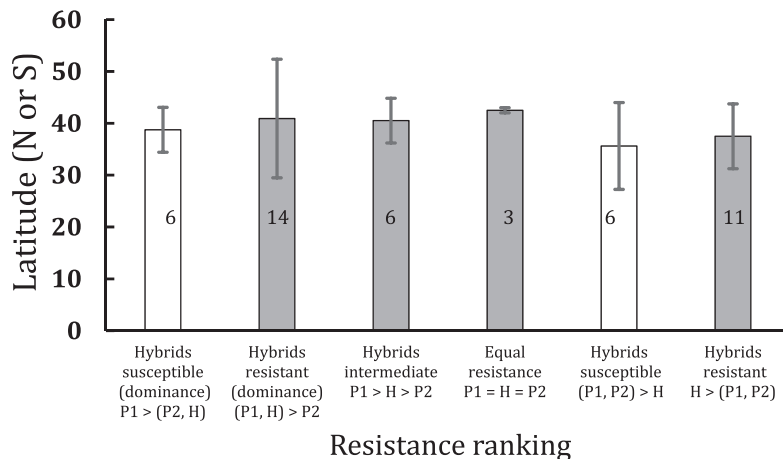
Fig. 4.   Average (±SD) latitude for classes of herbivore preference patterns for hybrids vs. parents based on literature review (see Appendix S1 for details). Numbers on histogram bars represent number of studies. If herbivores drive differentiation more frequently in the tropics than in temperate habitats, then the average latitude should be lower for systems with higher herbivore attack (numbers of individuals and/or associated damage) on hybrids than on parental types (susceptible with dominance, and susceptible categories, in white) than for other categories (in gray). "Intermediate" equals Whitham (1989)'s additive category. The mean latitudinal values among the classes are not significantly different (Kruskal–Wallis ranked sum test, $\chi^2 = 11.684$, df = 15, $P = 0.703$).

2002), and defenses are greater at low latitudes, for which there is some evidence (Coley and Aide 1991, Pennings et al. 2007, Marquis et al. 2012, Pearse and Hipp 2012). Finally in our review of herbivore attack in hybrid zones (Appendix S1), we find no current evidence that hybrids are more susceptible at lower latitudes (Fig. 4). This latter conclusion must be taken as a preliminary given that there have been so few studies in the tropics (see Appendix S1).

Conclusion

Ehrlich and Raven's hypotheses were published 50 yr ago in one of the most highly cited papers in evolutionary ecology. The two hypotheses—one, that herbivores have driven much of plant speciation, and two, that this process has been more active in the tropics—have not been examined in detail, much less tested. Perhaps this lack of attention is because these two hypotheses were presented without mechanisms or were lost in the larger message of the original thesis, namely that insect herbivores and plants have had reciprocal effects on each other's evolution. Indeed, if one were to include parasitoid speciation (Hawkins 2005) as dependent on the interactions between plants and insects, the statement that plant-insect herbivore coevolution has produced much of macroscopic organic diversity may be true.

The hypotheses are appealing, in that they are consistent with the long-held view that biotic forces in the tropics are particularly strong (Schemske et al. 2009). The difficulty is not a lack of evidence that herbivores can effect evolutionary change in their host plants (Marquis 1991). The key, instead, for speciation in sympatry, is finding a mechanism by which such

evolutionary changes also lead to reproductive isolation in their host plants, producing new lineages. For speciation occurring in parapatry or allopatry, the key is to demonstrate that herbivores are a main force driving differentiation rather than the myriad other factors that likely vary geographically.

We propose that two main mechanisms produce such reproductive isolation in conjunction with selection for increases in or changes in defense. There is currently evidence for the individual steps of each proposed mechanism. A prezygotic barrier to gene flow potentially can arise due to interactions between herbivores and pollinators, resulting the production of new species sympatrically. Studies of *Dalechampia* species and their herbivores and pollinators provide the best available example of a prezygotic scenario. However, some questions remain: How is intraspecific variation in defensive chemistry linked to differences in pollinator species identity? What is the geographic scale over which changes in herbivore and pollinator community composition occur in *Dalechampia*? In the case of *Dalechampia*, the herbivores are florivores. Can herbivores that feed on vegetative tissue (above- and/or belowground) also drive diversification?

Postzygotic barriers to gene flow can result in parapatric or allopatric speciation, and be driven by herbivores alone. Studies of incipient speciation in *Protium* in the Amazon basin (Fine et al. 2004, Lokvam et al. 2015) provide the best evidence for postzygotic mechanisms. Here *Protium* varieties differ markedly in defensive chemistry, they grow on soils that differ in nutrient availability and herbivore communities, and the impacts of these herbivores on plant growth vary by soil type and plant variety. The question arises as to how

different the soil types must be in order to produce tradeoffs. Furthermore, can other types of microhabitats (understory vs. light gap, riparian vs. *terra firma* forests, grassland vs. neighboring forest) also produce such tradeoffs?

Our main conclusion is that our understanding of how herbivores influence plant diversification is limited because we have not tested feasible mechanisms, as opposed to having done so and found the evidence lacking. The tools are available now for testing these hypotheses but will require expertise in natural history, pollination and herbivore ecology, plant genetics, plant systematics, and phytochemistry.

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CONCEPTS & SYNTHESIS

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Supporting Information

Concepts & Synthesis