

# Latex: A Model for Understanding Mechanisms, Ecology, and Evolution of Plant Defense Against Herbivory

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## Key Words

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## Abstract

Latex is a sticky emulsion that exudes upon damage from specialized canals in about 10% of flowering plant species. Latex has no known primary metabolic function and has been strongly implicated in defense against herbivorous insects. Here we review historical hypotheses about the function of latex, evidence that it serves as a potent defense, and the chemistry and mode of action of the major constituent defense chemicals and proteins across a diversity of plant species. We further attempt to synthesize the characteristics of latex as a coordinated plant defense system. Herbivores that feed on latex-bearing plants typically evade contact with latex by severing the laticifers or feeding intercellularly, or may possess physiological adaptations. Convergent evolution appears to be rampant both in plants with latex and insects that exploit latex-bearing plants. Because latex shows phenotypic plasticity, heritability, and macroevolutionary lability, it is an ideal system to study plant-herbivore interactions using evolutionary approaches.

## INTRODUCTION

**Mandibulate:** refers to insects that have mandibles for biting or chewing leaf tissue (as opposed to having a proboscis or stylet for sucking)

**Latex:** a milky suspension or emulsion of particles in an aqueous fluid, usually held under pressure in living plant cells (laticifers)

***Hevea brasiliensis* or Pará rubber tree (Euphorbiaceae):**

native to the Amazon, and perhaps the best-studied latex-producing species because of the economic importance of rubber

***Asclepias:*** a genus of herbaceous perennial plants with  $\approx 140$  species in the Americas and named for their exuding milky latex, commonly called milkweeds

About 10% of all flowering plant species (angiosperms) exude latex upon tissue damage and this latex has no known function in primary metabolism (in terms of plant resource acquisition and allocation) (Farrell et al. 1991, Hunter 1994, Lewinsohn 1991, Metcalf 1967). Over the past 20 years, a growing literature has emerged on latex, its biochemistry, and its ecological and evolutionary consequences. Both circumstantial and experimental evidence points to latex as a potent plant defense against mandibulate herbivores. Here we review various aspects of the chemical ecology and evolutionary biology of latex with special reference to plant-herbivore interactions. We use latex as a model to address general conceptually motivated questions that scientists might ask about any plant defense (Sidebar: A set of conceptually motivated questions addressing the mechanisms, ecology, and evolution of any plant defense trait).

Not unlike the blood of animals, when the tissues of latex-bearing plants are damaged, latex oozes out, becomes sticky when exposed to air, and quickly coagulates. Sometimes latex exudation can be remarkably abundant, like a squirt of toxic white glue, whereas at other times it may be difficult to detect because it is clear or barely exudes (Agrawal et al. 2008, Metcalf 1967, Shukla & Krishna Murti 1971). Latex is sometimes colored yellow, orange, or red, such as that of *Cannabis* (Cannabaceae). Latex is well known for its sticky properties, which have been used to produce rubber (from *Hevea brasiliensis* Euphorbiaceae and other species), chicle from *Manilkara* spp. (Sapotaceae) used in chewing gum, and lacquers from phenols in the latex of plants in the Anacardiaceae. Latex from various plant species contains bioactive compounds including alkaloids such as morphine in *Papaver* spp. (Papaveraceae); cardiac glycosides in *Asclepias* spp. (Apocynaceae); terpenes such as the sesquiterpene lactone, lactucin, from lettuce (*Lactuca* spp. Asteraceae); and digestive cysteine proteases in *Carica papaya* (Caricaceae) and *Ficus* spp. (Moraceae) (see section on Biochemistry and Mode of Action).

As evidenced from the above list of plant families with latex-bearing species, latex is extraordinarily common. Among flowering plants, over 20,000 species (from over 40 families in multiple lineages) contain latex (Farrell et al. 1991, Hunter 1994, Lewinsohn 1991, Metcalf 1967). Latex is found in dicotyledonous and monocotyledonous (e.g., Liliaceae) plants. This finding, that nearly 10% of families and species produce latex, implies that latex is a highly convergent trait (that is,

### A SET OF CONCEPTUALLY MOTIVATED QUESTIONS ADDRESSING THE MECHANISMS, ECOLOGY, AND EVOLUTION OF ANY PLANT DEFENSE TRAIT

What is the mode of action? Are the constituents that make up the defense redundant, additive, or synergistic? Is this defense tied into primary plant metabolism, protection from abiotic stress, energy storage, or waste? How specific are the effects of the defense against a diversity of attackers? Does this defense interact with predation? How do resources and abiotic environment modulate expression of the trait? Are there ontogenetic shifts in the expression of this trait? Is the defense inducible by herbivory? Which plant hormones regulate defense investment? Is there specificity in the elicitation of the induced response? How do herbivores cope with this defense? In what sorts of communities is this defense present, and what are the consequences of herbivory at the community level? Is there heritable variation for the expression of the defense? What maintains this heritable variation (allocation or ecological costs, spatial or temporal variation in the benefits)? How does this defense trait covary with other defense traits within the species? Do herbivores impose natural selection on this trait? How evolutionarily conserved is the defense in a clade of related plants? Is the defense adapted to particular populations or habitat types? Does this defense show phylogenetic patterns or trends? Any evidence for this defense as a key innovation?

has evolved independently multiple times) and that latex is likely encountered by many herbivore species. In addition, latex has been reported in mushrooms (e.g., *Lactarius* spp.), conifers (e.g., *Gnetum* spp.) and pteridophytes (Metcalfe 1967). Both in terms of absolute and proportional estimates, tropical plant families (and species overall) are more likely to produce latex than are temperate groups (Lewinsohn 1991). Indeed, some 14% of tropical plant species produced latex compared to 6% of temperate species, and this distribution is not independent of plant phylogeny (Lewinsohn 1991). Latex is phylogenetically conserved in the above-mentioned plant families, and these and other latex-bearing families may be overrepresented in the tropics.

## ANATOMY

Latex can be distinguished from resins, mucilages, and gums, which also exude upon tissue damage. Resins are typically composed of terpenoids and phenolics and delivered from intercellular spaces, sometimes called ducts (not from living cells like laticifers) (Langenheim 2003). For example, *Bursera schlechtendalii* stores toxic terpenes in pressurized resin ducts, which, following damage, can squirt over 1 m and may drench an herbivore and coat the leaf surface (Becerra 1994). Although the phloem primarily transports products of photosynthesis, in some plants such as *Cucumis* spp. (Cucurbitaceae), the pressurized phloem sap exudes upon damage and, like latex, gels into a sticky substance. Indeed, the sap of cucurbits additionally contains various plant defense compounds (Dussourd 1997, Dussourd & Denno 1991, McCloud et al. 1995). Latex tends to be more phytochemically diverse than resins, mucilages, and gums, and often contains complex mixtures of terpenoids, phenolics, proteins, and alkaloids (Langenheim 2003).

Laticifers take two main morphological forms (**Figure 1**). Nonarticulating laticifers are formed from single cells that often branch, but do not loop or reconnect (Dussourd & Denno 1991, Pickard 2008). This form is typified by the laticifers of the milkweeds (*Asclepias* spp.), which result from only 16 elongate cells that branch and spread through most above-ground tissues (Wilson 1986). Such remarkably long and multinucleate branching laticifers are also known from cytological studies of other species, such as *Jatropha dioica* (Euphorbiaceae), where 5 to 7 cells make up the entire laticifer network (Cass 1985). Articulating laticifers form loops and are often connected by perforations in the cell walls of neighboring laticifers. Articulating laticifers, such as those produced in the Asteraceae and Caricaceae, are produced by larger chains of cells that form net-like structures, and tend to deliver latex much more comprehensively to local tissues.

All plant parts can contain latex. The commonly examined tissues of latex-bearing plants are stem and leaf tissue. Indeed, we are not aware of latex-producing plants that do not exude the latex in stems and leaves. Exudation of latex in roots appears more variable. *Asclepias* species apparently do not exude latex from roots, although at least a few species have laticifers in root tissues; other species, such as those in the Asteraceae, exude copious latex from roots (Lucansky & Clough 1986; A.A. & K.K., personal observations). Latex exudation from reproductive tissues (buds, flowers, and fruits) is commonly observed, but like root latex, is far less studied than stem and leaf latex. Laticifers can transport latex and defensive substances upward of 70 cm to the damaged (exuding) points, as was demonstrated for the milkweed rubber vine *Cryptostegia grandiflora* (Buttery & Boatman 1976).

## HISTORY AND HYPOTHESES

Like many plant defenses, latex has been observed, described, and used by humans for thousands of years (Mahlberg 1993). Historically, latex was classified by its often opaque sticky exudation, and the propensity to coagulate upon exposure to air. As early as the 1600s, the term latex was used

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**Resin:** plant exudates common in conifers and some angiosperms (e.g., Anacardiaceae), rich in terpenoids and phenolics, and stored in intercellular spaces

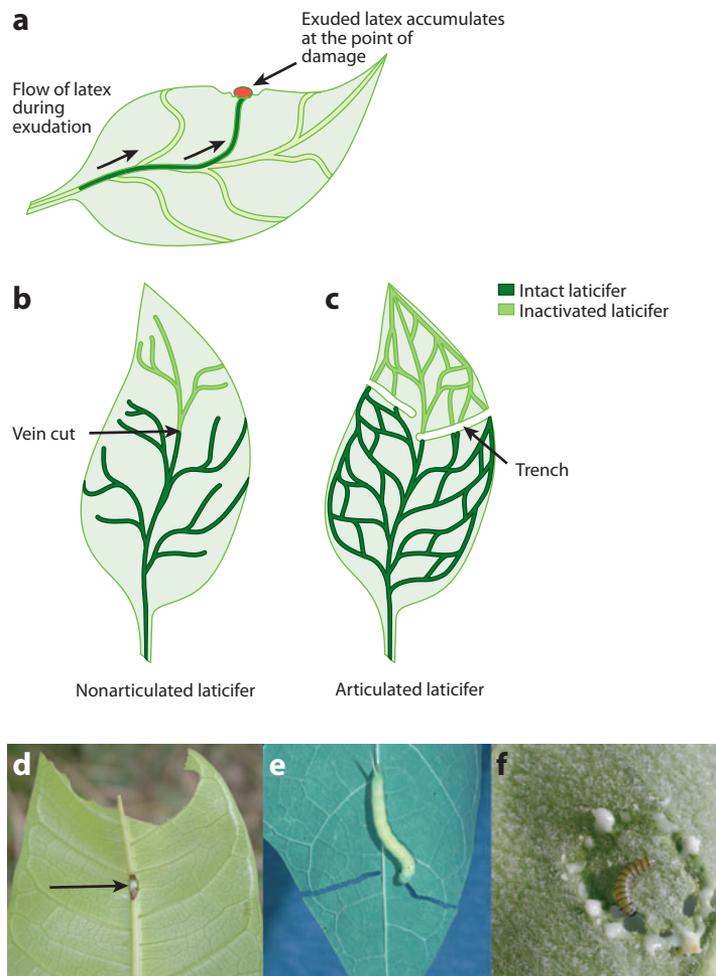
**Mucilage:** plant-produced sticky polysaccharides that are often clear and exude from the phloem following damage (common in cucurbits)

**Gum:** distinct from latex, resin, or mucilage; these water soluble polysaccharides exude from cellular cavities or bark (as in Rosaceous fruit trees)

**Laticifer:** an elongated cell with two main morphological types (articulated or nonarticulated) produced in any plant part, serving to transport latex

**David Dussourd:** American biologist at the University of Central Arkansas who has been the leading scientist unraveling the mechanisms of latex as a plant defense

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**Figure 1**

Latex exudes from the damaged points soon after the wounding by herbivores, and latex flow is caused by internal pressure. (a) The latex exists throughout the laticifer before insect attack (*dark green*) and concentrates at the point of damage (*red*). (b) Vein-cutting behavior by specialists feeding on nonarticulated laticifers and (c) trenching behavior by specialists feeding on articulated laticifers. (d) Vein-cut (*arrow*) made on a leaf of *Ficus virgata* (Moraceae, a wild fig with nonarticulated laticifers) by the larvae of *Cylestis thyodamas* (Nymphalidae) (Ishigaki Island, Okinawa, Japan); (e) larvae of *Erinnyx alope* (Sphingidae) trenching on *Carica papaya* (Caricaceae, papaya with articulated laticifers) (photo courtesy of David Dussourd); and (f) a first instar monarch butterfly larva *Danaus plexippus* employing a circle trench on milkweed with nonarticulated laticifers (although monarchs typically use vein cutting when larger, first instar larvae employ trenching, presumably because of their small size).

by English-speaking physicians, its function was analogized to lymphatic vessels of animals, and it was studied in several plant families (Mahlberg 1993). In a classic essay describing North American milkweeds, James (1887) apparently proposed the first defensive hypothesis for latex, “. . .it carries with it at the same time such disagreeable properties that it becomes a better protection to the plant from enemies than all the thorns, prickles, or hairs that could be provided. In this plant, so copious and so distasteful has the sap become that it serves a most important purpose in its economy.”

Experimentally, only a few years after James' essay, Kniep (1905) in Germany, published results of the first experiments to demonstrate latex as a resistance trait. He repeatedly damaged leaves of a plant in the Euphorbiaceae until latex no longer flowed from new cuts. Slugs readily ate such leaves, but refused to eat the leaves of intact plants that were not drained of their latex. Nearly a century later, Dussourd & Eisner (1987) showed that such disarming of the latex response, that is, severing of the laticifers, was routine maintenance before meals in most of the mandibulate herbivores of milkweeds. An additional smoking gun implicating the adaptive role of latex comes from the observations that upward of 30% of newly hatched monarch butterfly caterpillars (*Danaus plexippus*) die mired in latex of *Asclepias humistrata* (Zalucki & Brower 1992).

Other explanations for the production of latex have included functions involved in primary metabolism, namely storage and movement of plant nutrients, waste, and maintenance of water balance. Essentially none of these functions remain credible and none have any empirical support (Farrell et al. 1991, Hunter 1994, Mahlberg 1993). For example, carbohydrates in *Euphorbia* latex are unavailable to the plant, even when light starved (Nissan & Foley 1986). Although the costs of latex production have not been estimated for any plant species (see section on Evolution of Latex), the fact that latex is often highly concentrated with secondary metabolites, carbohydrates, and enzymes suggests that it is not a waste product. In *H. brasiliensis*, latex can be up to 60% dry mass rubber and 2% nitrogen (Shukla & Krishna Murti 1971); sugar-mimic alkaloids can make up to 18% of dry mass latex in mulberry, 100 times higher than the average concentration in leaves (Konno et al. 2006).

Studies of banana (*Musa* sp. Musaceae) indicate that the concentration of latex during exudation is correlated with the turgor pressure of the plant, and Milburn and colleagues (Kallarackal et al. 1986, Milburn et al. 1990) have suggested that latex may be important in maintaining turgor via osmoregulation. In particular, banana latex contains lutoids, which are transparent vesicles that compartmentalize various inclusions and have osmotic activity. Although a relationship between leaf turgor and latex concentration has also been found for *Nerium oleander* (Apocynaceae) (Downton 1981), no studies have shown that specific attributes of the latex actually function to help maintain plant water balance. It is more likely that latex simply responds to turgor pressure.

## EVIDENCE FOR LATEX AS A DEFENSE

Various forms of evidence, from the observational to the experimental and comparative, have been accumulated in support of latex as a plant defense against herbivory. Nonetheless, few studies to date have linked variation in plant production of latex to plant fitness (Agrawal 2005a). In the strict sense, a plant defense is any trait that improves the fitness of plants in the presence (but not the absence) of herbivores (Karban & Baldwin 1997). Thus, although we have assumed that latex is defensive (and hence adaptive for the plant), most studies have focused on latex as a trait that reduces herbivory or the preference or performance of herbivores.

As a follow-up to the classic experiments of Kniep described above, Zalucki and colleagues have conducted several studies demonstrating that depressurizing the latex of milkweeds (*Asclepias* spp.) increased the fitness (survival or growth) of specialized monarch butterfly caterpillars (*Danaus plexippus*). By partially severing a leaf's petiole, the flow of latex to that leaf can be essentially stopped without altering the turgidity of the leaf. This treatment substantially improved fitness of monarchs on four milkweed species with high latex flow, but had relatively minimal effect on larvae when four milkweed species that produce less latex were treated (Zalucki et al. 2001b; Zalucki & Malcolm 1999). These experiments provide convincing evidence for a role of latex in resistance to a specialist herbivore. Konno et al. (2004) further showed that washing papaya and fig leaves free of their latex made them acceptable to herbivores that typically perish on intact leaves.

Several studies have added latex to artificial diets or painted leaves with latex to assess its role in resistance. For example, latex of the milkweed *Hoodia gordonii* proved deterrent to larval feeding and adult oviposition by generalist cabbage loopers (*Trichoplusia ni*) (Chow et al. 2005). Latex of a resistant variety of lettuce *Lactuca sativa* ‘Valmaine’ almost completely inhibited feeding of *Diabrotica balteata* beetles when painted on leaves of a favored food (lima bean leaves) (Huang et al. 2003). Nonetheless, latex from a different, susceptible variety of lettuce ‘Tall Guzmane’ was not deterrent (Sethi et al. 2008). This study implicated chemical compounds in the resistant latex (a moderately polar fraction of the latex) that were not present in the latex of the susceptible lettuce variety.

Quantitative genetic evidence demonstrates that latex is an important component of resistance to herbivores. For example, in an analysis of 96 genetic lines of sweet potato (*Ipomoea batatas*), latex exudation was found to vary nearly 20-fold (Data et al. 1996). Application of this same latex to root cores of sweet potato reduced feeding and oviposition by a specialist weevil, *Cylas formicarius*. Full-sibling genetic families of the common milkweed (*Asclepias syriaca*) can vary over fourfold in their latex production when grown in a common garden (Agrawal 2005a, Van Zandt & Agrawal 2004). This variation predicted growth of the specialist chrysomelid beetle *Labidomera clivicollis* (Van Zandt & Agrawal 2004), abundance of a cerambycid beetle *Tetraopes tetraophthalmus* (Agrawal 2004), abundance and oviposition by a curculionid stem weevil *Rhyssomatus lineaticollis* (Agrawal & Van Zandt 2003), and overall community composition (Agrawal 2005a). Nonetheless, neither genetic variation in latex within one species nor variation in latex across 24 milkweed species was a good predictor of monarch caterpillar larval growth (Agrawal 2005a, Agrawal & Fishbein 2006).

Although the weight of evidence suggests that latex is defensive, and no strong alternative hypotheses have stood the test of time, the fitness benefits of latex production for plants have not been well quantified (see Agrawal 2005a and section on Evolution of Latex).

## BIOCHEMISTRY AND MODE OF ACTION

Plant latex, exudates (including phloem sap), and resins contain various secondary metabolites and proteins, often in concentrations that are much higher than in leaves. Indeed, the latex of most species contains a diversity of biologically active compounds. Many of these compounds provide resistance to herbivores via toxicity or antinutritive effects, whereas others are involved in the stickiness that can mire insect herbivores. Several of these defense-related components (e.g., rubber, cysteine protease, alkaloids, etc.) appear in latex of distant phylogenetic groups, suggesting common functions and convergent evolution. Below we survey the common constituents of latex, their mode of action, and possible biological effects on herbivores.

### Secondary Metabolites

**Rubber.** Rubber (*cis*-1,4-polyisoprene) is a terpenoid found in the latex of many plant species, across some 300 genera and 8 plant families (Bushman et al. 2006, Metcalf 1967, Mooibroek & Cornish 2000). Both the stickiness and typically white color of latex are often derived from rubber particles dispersed in the fluid. Rubber can make up a high concentration of fresh latex (e.g., *H. brasiliensis* 44.3%, *Ficus* spp. (Moraceae) 15–30%, *Alstonia boonei* (Apocynaceae) 15.5%, *Parthenium argentatum* (Asteraceae) 8%) (Mooibroek & Cornish 2000). Given its high concentrations, uniform structure, and widespread distribution among unrelated plant families, rubber in latex has likely convergently evolved, suggesting an important adaptive function. At present, it is generally accepted that the primary functional role of rubber in latex is to produce stickiness that entraps whole insects (Dussourd 1993, 1995) or mires their mouthparts (Dussourd & Eisner 1987).

Additionally, rubber is involved in sealing leaf wounds, which should prevent further drainage of latex and may prevent infection by pathogens. However, the mechanisms of these effects (even for stickiness) are not well documented. We propose that the stickiness of latex may be caused by at least three factors: (a) elasticity of *cis*-polyisoprene, (b) coagulation of rubber particles, and (c) adhesiveness of rubber particles to the surfaces of insects.

**Alkaloids.** Alkaloids are basic (as opposed to acidic) natural products containing nitrogen, many of which are toxic and typically do not have a primary function in plants. Alkaloids are produced by a variety of animals, microorganisms, and plants and have been reported from the latex of many species, sporadically distributed among angiosperm families, including Papaveraceae and Moraceae. For example, isoquinoline alkaloids such as chelidonine, sanguinarine, and copticine can total nearly 20% fresh mass of the latex in *Chelidonium majus* (Tomè & Colombo 1995). Sanguinarine affects neurotransmission by inhibiting choline acetyl transferase, various neuroreceptors, and also DNA synthesis (making it toxic to insects and vertebrates alike) (Schmeller et al. 1997). Morphine is produced in the latex of *Papaver somniferum* (opium poppy) up to 5% fresh mass latex (Itenov et al. 1999). It was recently found that synthetic enzymes involved in the early stage of alkaloid synthesis are localized in parenchymal cells surrounding laticifer cells and that those involved in the late stages of synthesis were localized inside the laticifer (Samanani et al. 2006, Weid et al. 2004).

Recently, sugar-mimic alkaloids were found in the latex of mulberry species (*Morus* spp., Moraceae), and occurred up to 2.5% fresh mass (18% dry mass) in latex (Konno et al. 2006). Sugar-mimic alkaloids, also known as imino sugars, are potent inhibitors of various glycosidases and sugar-metabolizing enzymes (Asano et al. 2000). These compounds show toxicity and growth retardation in insects by inhibiting sucrase in the midgut and trahalase in various other tissues, which results in the inability to uptake sucrose and utilize trehalose (Hirayama et al. 2007).

**Cardenolides.** Cardenolides, or cardiac glycosides, inhibit  $\text{Na}^+/\text{K}^+$ -ATPases, which are important for maintenance of electric potential in most animal cells, making them remarkably toxic to a wide array of animals (Malcolm 1991). Latex of many plants in the Apocynaceae contains cardenolides, and these range from trace amounts up to 30% dry mass of latex (Malcolm 1991, Seiber et al. 1982). Additionally, latex of *Antiaris toxicaria* (Moraceae) in tropical Southeast Asia contains cardenolides (toxicariosides), which have been used as dart poisons (Carter et al. 1997). Cardenolides have also convergently evolved in a few other plant families (e.g., Brassicaceae, Celastraceae, Fabaceae), but in these cases they are not associated with latex (Malcolm 1991). In neuronal cells, cardenolides impact neurotransmission by binding to the amino acid asparagine at position 122 in the extracellular region of  $\text{Na}^+/\text{K}^+$ -ATPase, which is present in most animals including humans and *Drosophila* (Holzinger et al. 1992, Holzinger & Wink 1996).

Most cardenolide-containing plant species produce a diversity of compounds, due in particular to the different chemical structures of the glycosides in the molecule. Differences in the polarity of cardenolides, in particular, have been linked to differential absorption in the animal body, and thus with potentially differential toxicity. For example, nonpolar digitoxin is almost completely absorbed, irrespective of where it is administered to insects; conversely, ouabain, a highly polar cardenolide, is intestinally absorbed quite slowly (Malcolm 1991). Nonetheless, the adaptive significance of cardenolide diversity is unknown. Virtually nothing is known about where the cardenolides are produced and how they are transported into the latex (Groeneveld 1999). Cardenolides have no known functions in plants other than defense.

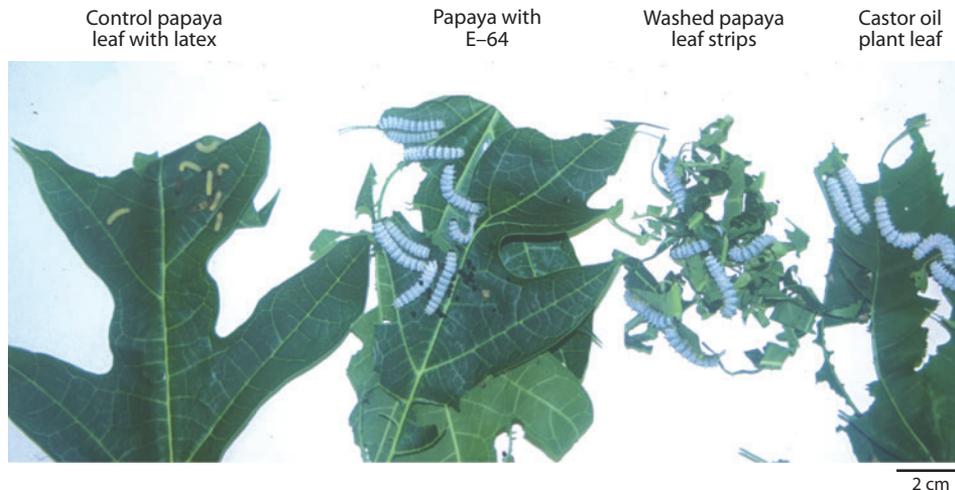
**Terpenoids.** Terpenoids (including rubber) are an extremely diverse group of carbon-based compounds that are derived from five-carbon isoprene units. Terpenoids likely have many functions in plants, including pollinator attraction, defense, and roles in primary metabolism (e.g., carotenoids that provide additional pigments for harvesting light energy), and can be produced abundantly in latex. The latex of *Lactuca sativa* (cultivated lettuce) contains several sesquiterpene lactones (SL), including lactucin and the total SL concentration in the bolting stage of lettuce reached 147.1 mg/ml latex (Sessa et al. 2000). In particular, the concentration of lactucopicrin oxalate in latex was over 1000 times higher in concentration than that in foliar tissue. In addition to those constitutive SLs, lettuceenin A is induced in latex by fungal and bacterial challenge. Lactucopicrin and 8-deoxylactucine are known to deter feeding by locusts (Rees & Harborne 1985), and lactucin triggers leaf-trenching by *Trichoplusia ni*, a facultatively trenching caterpillar (Dussourd 2003). These SLs have some antifungal activity against the pathogenic, *Cladosporium berbarum*; and lettuceenin A, which is induced in latex by microorganisms, strongly inhibited the growth of *Cladosporium berbarum* (Sessa et al. 2000).

The latex of some Euphorbiaceae, such as *Euphorbia biglandulosa*, contains diterpenes such as phorbol and its derivatives (Noack et al. 1980). These compounds have toxicity against insects and mammals, have tumor-promoting activity, and cause skin inflammation (Gershenson & Croteau 1991). Further, triterpenoids are reported as the major components of the latex of some *Euphorbia* spp. (Mazoir et al. 2008).

**Phenolics.** Phenolics are a huge group of multifunctional carbon-based secondary metabolites produced by the shikimate pathway that include tannins, lignins, and flavonoids. Latex of the sweet potato, *I. batatas* (Convolvulaceae) contains high concentrations of hexadecyl, octadecyl, and eicosyl esters of *p*-coumaric acids (Snook 1994). The overall concentration of *p*-coumarate esters exceeded 3% fresh vine latex and 10% root latex of the variety “Jewel” (Snook 1994). The concentrations of (*Z*)-isomers of C<sub>16</sub>, C<sub>18</sub>, C<sub>20</sub> coumarates inversely correlated with the acceptability by weevils, indicating that (*Z*)-coumarate esters may participate in the defense of sweet potato against insect herbivores (Snook 1994). Additionally, the latex-like resin of *Rhus* spp. (lacquer plant, poison ivy, etc. in Anacardiaceae) are well known to contain urushiol, a catechol with a long carbon chain rich in double bonds and a compound known to cause strong skin irritations (Dawson 1954).

## Proteins

**Proteases.** Proteases are enzymes that cleave protein and are found in all living organisms. Various types of proteases are found from latex of plants belonging to diverse phylogenetic clades. For example, cysteine proteases are reported from latex of plant families such as Caricaceae, Moraceae, Apocynaceae (Kimmel & Smith 1954; Arribère et al. 1998; Sgarbieri et al. 1964), and serine protease from Moraceae, Euphorbiaceae, Apocynaceae, Convolvulaceae (Arima et al. 2000; Tomar et al. 2008). The latex-like resin exudates of mango, *Mangifera indica* (Anacardiaceae), contain both serine and cysteine proteases (Saby et al. 2003). In spite of the abundance and frequent occurrence of proteases in plants, an adaptive role of these compounds for plants was not suggested until recently. Direct evidence for the involvement of cysteine proteases in plant resistance against herbivores came from experiments showing that the strong toxicity of papaya and wild fig (*Ficus virgata*) leaves against the Eri silkworm, *Samia ricini*, and the cabbage worm, *Mamestra brassicae*, disappeared when latex was washed out of the leaves or when E-64, a cysteine protease-specific inhibitor, was painted on the surface of leaves (Konno et al. 2004) (**Figure 2**). These results demonstrated that cysteine proteases in papaya (papain) and fig (ficin) latex are a crucial part of



**Figure 2**

Defensive activity of papain, a cysteine protease from latex of *Carica papaya*. (a) Second instar Eri silkworm, *Samia ricini*, fed intact (clipped from the petiole) leaves containing latex died; (b) leaf toxicity was lost after painting E-64, a cysteine protease-specific inhibitor, on the leaf surface, or (c) removing the latex by washing it out of leaf strips; (d) larvae on these treated leaves grew as well as others that were fed their natural host plant, *Ricinus communis* (modified from Konno et al. 2004). The photo was taken on day 4.

plant resistance (**Figure 2**). The protease activity in papaya latex is 20 times higher than the lethal concentration of papain to nonadapted herbivores, and nearly 200 times higher in concentration in latex compared to that in leaves (Konno et al. 2004).

Proteases are digestive (that is, they break apart protein) and are commonly found in animal guts. Thus, their role as a plant defense appears to be a remarkable turn on the plant-insect interactions, essentially plants eating the insect! Nonetheless, the mechanisms of protease toxicity have not well studied except for Mir1-CP, which is toxic to insects and accumulates at the site of larval feeding in maize lines resistant to caterpillars (Pechan et al. 2000). Here, the cysteine proteases degrade the peritrophic membrane of the insect midgut, which consists of proteins and chitin (Pechan et al. 2002). The observation that the dead bodies of caterpillars mired in latex of papaya, fig, and milkweed turn black and soft (A.A. Agrawal & K. Konno, unpublished data) indicates that all tissues of insects are a potential target of digestion by proteases in latex.

**Protease inhibitors (PIs).** PIs are thought to function as antinutritive secondary metabolites by binding to proteases and preventing the digestion of protein. Trypsin (serine protease) inhibitors are found in latex of *Ficus carica* (Kim et al. 2003) and *Carica papaya* (Azarkan et al. 2004). Gene expression of trypsin inhibitors is also in the laticifers of *H. brasiliensis* (Han et al. 2000). Furthermore, the latex-like ploem sap of *Cucurbita maxima* (pumpkin, Cucurbitaceae), contains various types of protease inhibitors including trypsin, chymotrypsin, and cysteine or aspartic inhibitors (Kehr 2006, Walz et al. 2004). Protease inhibitors inhibit proteolysis and utilization of proteins, and their defensive roles against herbivores and fungi are well-established in many plants without latex (Ryan 1990, Zhu-Salzman et al. 2008). Trypsin inhibitor, a class-II chitinase and a glutaminyl cyclase, is absent from latex of undamaged leaves, but was strongly induced in latex after damage (Azarkan et al. 2004).

**Lectins and hevein-like chitin-binding proteins.** Lectins are carbohydrate-binding proteins that have affinity with specific sugar moieties, which often have toxic activities against animals including insects (Van Damme et al. 1998). Several types of lectins have been found in latex from Euphorbiaceae, Moraceae, Apocynaceae, and phloem sap from Cucurbitaceae. Of these, hevein, the major latex protein from *H. brasiliensis* is important in the agglutination of rubber particles (Gidrol et al. 1994), and its m-RNA is induced by wounding (Broekaert et al. 1990). Upon exposure to air, hevein binds to receptor proteins and cross-linked rubber particles, thereby causing coagulation of latex.

The mechanisms of coagulation have also been studied in the latex-like phloem sap of cucurbits (Read & Northcote 1983, Kehr 2006). After exudation, two major proteins in the sap, PPI and PPII, coagulate and form a fibrous structure (both proteins are soluble in the intact phloem). When in contact with air, PPI and PPII molecules are crosslinked through S-S bonds, formed among cysteine residues. Coagulation of cucurbit phloem sap not only stops exudation but also glues mouth parts of beetles and can inhibit feeding (McCloud et al. 1995).

The chitin-binding MLX56 proteins from *Morus* latex are highly toxic to many caterpillars including the cabbage worm, *Mamestra brassicae* (Wasano et al. 2009). Proteins (including chitin-binding and chitinolytic proteins and cysteine proteases) extracted from the latex of the milkweed *Calotropis procera* were shown to have insecticidal action against four different crop pests when incorporated into artificial diets (Ramos et al. 2007). Chitin-binding proteins with hevein-like domains, such as the wheat germ lectin, are toxic and inhibit the synthesis of the insect gut peritrophic membrane (Hopkins & Harper 2001, Van Damme et al. 1998).

**Chitinases.** Chitinases, enzymes that degrade chitin (important components of insects' gut peritrophic membrane), are widely found in plant latex from several plant families including Caricaceae, Moraceae, and Euphorbiaceae (Howard and Glazer 1969; Glazer et al. 1969; Lynn & Clevette-Radford 1987). Because chitin is the major constituent of the cell wall of fungi, it is reasonable to assume that chitinases protect the leaves from infection by pathogenic fungi as well. Expression of chitinases in the latex of *F. carica* and *C. papaya* increases in response to wounding or treatment with jasmonic acid (the plant hormone involved in signal transduction of plant responses to herbivory) (Azarkan et al. 2004, Kim et al. 2003). Chitinases from insect origins show toxic effects on other insects when orally ingested (Kramer & Muthukrishnan 1997, Kabir et al. 2006), suggesting that chitinases in latex may have a defensive role. Nonetheless, the toxic effects and defensive roles of plant chitinases are not well established, except for chitinases of poplar trees (not in latex), which are induced in response to herbivory and provide protection against subsequent attack (Lawrence & Novak 2006).

**Oxidases.** Polyphenol oxidase (PPO) and peroxidase (POD) are common plant oxidases reported from Euphorbiaceae, Moraceae, and Anacardiaceae (Saby et al. 2003, Wititsuwannakul et al. 2002). PPOs and some PODs are regarded as plant antiherbivore defense proteins, because they oxidize mono- or di-hydroxyphenolics that are ultimately converted in *o*-quinones, which then covalently bind to amino acids such as cysteine and lysine, making them inaccessible, and decrease the nutritive value of leaf protein (Felton et al. 1992, Zhu-Salzman et al. 2008). The frequent browning of latex upon exposure to air in many plant species suggests that PPOs and PODs may be widely distributed in latex. Proteomic analyses of phloem sap exuded from cucumber, *Cucurbita sativa* and pumpkin, *Cucurbita maxima*, revealed abundant lipoxygenases (LOX) (Walz et al. 2004). LOXs are implicated as defense proteins since they are often induced by wounding or jasmonic acid, and since hydroperoxides formed by the oxidation of linolenic/linolenic acids by LOXs may

react with amino acids, in addition to the loss of fatty acids essential for insects (Felon et al. 1994, Zhu-Salzman et al. 2008).

**Others.** In addition to the above-described proteins that were reported from many plant groups, some latex proteins are confined to specific plant taxa and have been suggested to be involved in plant defense. These compounds include phosphatase in Euphorbiaceae (Lynn & Clevette-Radford 1987); lipase in Caricaceae, Euphorbiaceae, Apocynaceae (Fiorillo et al. 2007, Gandhi & Mukherjee 2000, Giordani et al. 1991); glutamyl cyclase in Caricaceae (papaya) (Azarkan et al. 2004, Zerhouni et al. 1998); and gum arabic glycoprotein, a high-molecular-weight, hydroxyproline-rich arabinogalactan-protein found from exudates of *Acacia senegal* (Fabaceae) (Goodrum et al. 2000). Finally, linamarase in cassava leaves and latex is a  $\beta$ -glucosidase that specifically degrades linamarine, also present in the leaves and roots of the same plant, and results in the production of hydrogen cyanide that is toxic to most organisms. The linamarase activity reported from the latex of cassava was more than 300-fold higher than that in its leaves (Nambisan 1999).

## HERBIVORE ADAPTATIONS FOR FEEDING ON LATEX-BEARING PLANTS

Many herbivorous insects, particularly specialists, have adaptations to cope with the toxins and antinutritive agents in the leaves (and latex) of plants. For example, larvae of monarch butterflies specialized in feeding on milkweeds exuding cardenolide-containing latex have evolved  $\text{Na}^+/\text{K}^+$ -ATPases insensitive to cardenolides (Holzinger & Wink 1996), and this ability was convergently evolved in other insect groups such as *Chrysochus* beetles feeding on *Apocynum* (Labeyrie & Dobler 2004). The silkworm, *Bombyx mori*, a specialist feeding on mulberries (*Morus* spp.) exuding latex containing sugar-mimic alkaloids, has evolved sucrase and trehalase insensitive to sugar-mimic alkaloids (Daimon et al. 2008, Hirayama et al. 2007). Below we focus on behavioral tactics that herbivores employ to reduce exposure to both the sticky physical aspect of latex and the concentrated phytochemicals in latex.

Many insects have evolved behaviors to inactivate the pressurized delivery system of laticifers. Since the laticifer system relies upon the ability to transport defense substances (**Figure 1**), their functions are lost when the transport routes are disrupted (Dussourd 1993, 1999; Dussourd & Denno 1991, 1994; Dussourd & Eisner 1987). The loss of function is especially pronounced in nonarticulated laticifers (branching type) without bypassing loops (**Figure 1**). Cutting of the laticifers at a single location upstream of feeding can deactivate all downstream activity (**Figure 1**). Many mandibulate herbivores of latex-bearing plants with nonarticulated laticifers accordingly engage in vein-cutting behavior (**Figure 1**) (Dussourd 1993, Dussourd & Denno 1991, Dussourd & Eisner 1987). Plants with articulated laticifers (net or web type) (**Figure 1**) are better protected from herbivory because, even when insects cut veins, there are circuitous routes for latex to go downstream of the cut. Specialists feeding on leaves with articulated laticifers typically show a behavior called trenching (**Figure 1**), in which insects cut a leaf-wide trench or circle trench (**Figure 1**) (Dussourd 1993; Dussourd & Denno 1991, 1994). Whether herbivores employ vein-cutting or trenching corresponds well to the types of laticifer (that is, nonarticulate or articulated) of their host plants (Dussourd & Denno 1991).

The effects of these behaviors are quite evident from experiments showing that natural or artificial trenching or vein-cutting on latex-exuding leaves can reduce the amount of exuding latex and render leaves edible to herbivores not typically adapted to these plants (Dussourd & Denno 1991, 1994; Dussourd & Eisner 1987, Zalucki & Brower 1992). Just as latex is a widely-distributed

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**Trenching or vein-cutting:** the behavior of deactivating pressurized laticifers by insects, after which they typically feed on distal tissues free of latex exudation

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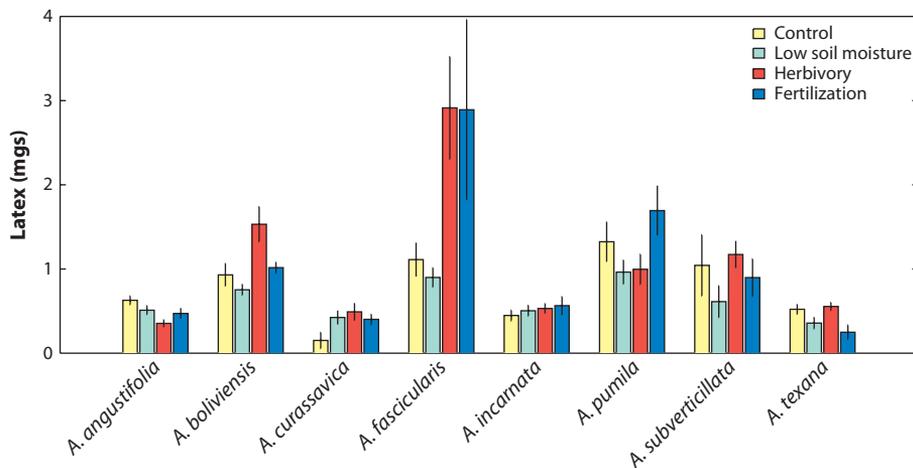
trait and is likely to have evolved independently multiple times, so too are trenching and vein-cutting behaviors. For example, vein-cutting is observed in Orthoptera (Tettigoniidae), Coleoptera (Cerambycidae, Chrysomelidae, Curculionidae), and Lepidoptera (Arctiidae, Gelechiidae, Noctuidae, Nymphalidae, Pyralidae), and trenching is observed in Coleoptera (Coccinellidae, Chrysomelidae) and Lepidoptera (Noctuidae, Nymphalidae, Sphingidae) (Dussourd & Denno 1991; A.A. Agrawal & K. Konno, personal observations). Such convergence is evident even on single host plants. Indeed, on common milkweed (*Asclepias syriaca*) in eastern North America, vein-cutting is commonly exercised by Arctiidae, Cerambycidae, Chrysomelidae, Curculionidae, and Nymphalidae (Dussourd & Eisner 1987, Fordyce & Malcolm 2000).

Not all herbivores on laticiferous plants trench or cut veins. For example, adult *Chrysochus auratus* (Chrysomelidae) feed on latex-rich *Apocynum* spp. and show population variation in whether or not they cut veins (Williams 1991; A. Agrawal, personal observations). Other herbivores, such as the milkweed leaf miner (*Liriomyza asclepiadis* Diptera), apparently feed without coming into contact with latex (Agrawal 2005a). Most sap suckers (Hemiptera) similarly do not contact latex because of their intercellular feeding, and thus have no obvious adaptations for feeding on latex-bearing species. *Asclepias syriaca* is host to at least five hemipterans (three aphids and two lygaeid bugs) (Agrawal 2005a, Smith et al. 2008; A.A. Agrawal, personal observations). Nonetheless, latex can occasionally entrap and kill hemipteran sap feeders, as was demonstrated for aphids and whiteflies on lettuce (Dussourd 1995).

Trenching and vein-cutting is a phenotypically plastic behavior. Many species will not engage in the behavior if feeding on an already depressurized leaf. It has been recently found that trenching and vein-cutting behavior in insects is also specifically triggered by compounds in latex and exudates (Dussourd 1997, 2003; Helmus & Dussourd 2005). The cabbage looper, *Trichoplusia ni*, cuts trenches on plants that produce exudates such as *Lactuca sativa* (latex), parsley (*Petroselinum crispum*, Apiaceae, oil from oil ducts), cucumber (*Cucumis sativus*, Cucurbitaceae, exudates from phloem), and cardinal flower (*Lobelia cardinalis*, Campanulaceae, latex), but it does not trench on plantain (*Plantago lanceolata*, Plantaginaceae), which does not have an exudate. When exudates from the above exuding species were applied orally to the cabbage looper beforehand, the loopers trenched on plantain leaves (Dussourd 1997, 2003). The triggering stimulants include lactucin (a sesquiterpene lactone) from lettuce latex, myristicin (a phenylpropanoid) from parsley oil, and lobeline (an alkaloid) from cardinal flower. Each of these compounds is known or suspected to be toxic; in other words, a diverse set of toxins are the cues used to induce trenching. The trenching stimulants from cucumber phloem exudates, however, are still unknown, but are certainly not cucurbitacins (Dussourd 2003, McCloud et al. 1995). Similarly, toxic furanocoumarins from parsley do not trigger trenching (Dussourd 2003). Finally, monarch caterpillars, specialists of milkweeds, do not initiate vein-cutting in response to cardenolides; some other nonproteinaceous and non-adhesive fraction of milkweed latex induces trenching, although the specific compounds have not been identified (Helmus & Dussourd 2005).

## PHENOTYPIC PLASTICITY OF LATEX PRODUCTION

Several lines of evidence suggest that latex production in plants is phenotypically plastic (that is, responsive to environmental conditions). For example, work on the rubber tree *H. brasiliensis* and sweet potato (*I. batatas* Convolvulaceae) shows that light levels, drought, and soil moisture conditions determine the amount of latex production (Data et al. 1996, Raj et al. 2005). In a recent study of several *Asclepias* species, we have shown that soil moisture, soil fertility, and leaf herbivory can all affect latex production (Figure 3). Several important results emerge from these data. First, closely related species vary nearly tenfold in their constitutive latex production. Second, all three



**Figure 3**

Impacts of environmental conditions (soil moisture, leaf herbivory by monarch caterpillars, and fertilization) on the production of latex (mean  $\pm$  SE) in eight milkweed species. Latex was measured on plants grown from seed in a common growth chamber environment by clipping the tip of a leaf and measuring the fresh mass of exuded latex. These eight species are close relatives, all in the Series Incarnatae of the genus *Asclepias*. Data from S.C. Cook, A.C. Erwin, M. Fishbein & A.A. Agrawal, unpublished manuscript.

environmental factors examined had considerable effects on latex production, but the influence was reversed in different species. For example, although herbivory reduced latex production in *A. angustifolia*, it substantially induced latex production in several other species. Third, the rank order of species for their latex production can be altered by environmental conditions. Thus, both the biotic and abiotic environment can have a strong influence on latex exudation.

Despite the fact that essentially nothing is known about the adaptive value of phenotypic plasticity in latex production, the variation observed in **Figure 3** is suggestive. For example, the nearly threefold increase in latex exudation following monarch herbivory in *A. fascicularis* compared to no indication of induction in several other species (including those with equivalent constitutive latex production) is suggestive of some adaptive function. In more recent work we have demonstrated that jasmonic acid application is sufficient as an inducer of latex in some *Asclepias* species (e.g., *A. fascicularis*) (S. Rasmann & A.A. Agrawal, unpublished data). This indicates that the near-universal plant hormone, jasmonic acid, which is responsible for the induction of sundry chemical defenses, trichomes, and extrafloral nectar throughout the angiosperms (Agrawal 2005b), is sufficient by itself to elicit an increase in latex exudation. In the rubber tree *H. brasiliensis*, jasmonic acid application increases the number of laticifers, and hence latex flow, in saplings (Hao & Wu 2000). Finally, there is some evidence for specificity in the induced latex response of *A. syriaca*. We reported that herbivory by specialist leaf beetles (*Labidomera clivicollis*) resulted in greater induction of latex compared to similar levels of herbivory imposed by monarch caterpillars (Van Zandt & Agrawal 2004). In addition to phenotypic plasticity in the amounts of latex being exuded following damage, the many concentrated secondary plant compounds in latex may also be plastically expressed (see section on Biochemistry and Mode of Action).

There is additionally an ontogenetic and qualitative component to plasticity in latex exudation. Young sweet potato vines (*I. batatas*) produced up to fourfold more latex and had less weevil feeding damage than older more mature portions of the vine (Data et al. 1996). A slightly different pattern has recently been observed for common milkweed (*A. syriaca*), with young leaves showing

equal levels of constitutive latex exudation as that of older leaves, but only the former showing inducibility following herbivory (R. Bingham & A.A. Agrawal, unpublished data). In addition to phenotypic plasticity in the quantity of latex exudation, the quality of latex is also plastic. For example, several defense-related proteins potentially involved in plant defense, such as chitinase, protease inhibitors, and glutaminyl cyclase, were induced in latex by damage of papaya leaves (Azarkan et al. 2004).

## EVOLUTION OF LATEX

From a microevolutionary perspective, the Darwinian criteria necessary for a trait to evolve by natural selection are that it must exhibit heritable variation that influences plant fitness. The only data available on heritability and selection (that is, differential fitness for varying phenotypes) on latex come from a field common garden study of *A. syriaca* (Agrawal 2005a). Over two years, the heritability was estimated in 23 full-sibling families, and was 0.161 in the first year, and 0.368 in the second year. In the same set of plants, latex exudation more than doubled in the second year of growth compared with that in first-year seedlings, indicating a strong ontogenetic component to latex production. In the second year of growth, genetic families from a single population showed over fivefold variation (between 0.8–4.5 mg latex exuded upon leaf damage). The relatively low, but still statistically significant, heritable variation is consistent with a strong environmental influence on latex production discussed above.

The genetic correlation between latex exudation and plant fitness from the study of *A. syriaca* revealed marginal evidence for directional natural selection to increase latex production (selection gradient  $\beta = 0.285$ ,  $P = 0.067$ ) (Agrawal 2005a). Given that this study was conducted in the field with ambient levels of herbivory, we conclude that any costs of latex were apparently outweighed by benefits associated with reduced herbivory. No other estimates of heritability, costs, or benefits of latex production for plants exist to our knowledge. The relative ease with which latex exudation can be quantified makes it an exceptionally attractive system for such studies. One potential influence on the adaptive evolution of latex is genetic correlations with other traits that affect fitness. For *A. syriaca*, however, latex was not genetically correlated with plant production of foliar cardenolides, trichomes, toughness, or nitrogen content (Agrawal 2005a).

Latex production has been implicated as a key innovation that has spurred adaptive radiation in plants. Across the angiosperms, Farrell et al. (1991) showed that latex-bearing plant clades were significantly more species rich than were sister clades lacking latex (13 of 16 pairs showed this pattern). Across more closely related species, the dynamics of trait evolution can be inferred in a phylogenetic context. In some groups such as the Asteraceae, Apocynaceae, Euphorbiaceae, and Sapotaceae, many if not most species produce latex, and this is likely to be the ancestral condition and to be evolutionarily conserved. Conversely, in other plant families (e.g., Aceraceae, Fabaceae, Salicaceae), latex may be derived and evolved in isolated taxa. Few systematic studies have examined the evolution of latex within clades.

Within *Asclepias* species, we have shown a pattern of phylogenetic decline in latex production as the genus diversified (Agrawal & Fishbein 2008, Agrawal et al. 2008). This pattern could be interpreted in two ways. First, reduced latex production is apparently associated with an increased diversification rate (within *Asclepias*). Alternatively, there could simply be evolution toward phenotypic declines in latex production within the group, and this may have little to do with diversification rate per se. It has been hypothesized that there may be selection for a macroevolutionary reduction of defense traits when plants are associated with a herbivore fauna dominated by specialists that can inactivate resistance mechanisms such as latex (Agrawal & Fishbein 2008, Agrawal et al. 2008).

Trait correlations in comparative analyses that are independent of phylogenetic history reveal either a strong physiological constraint that is unbreakable, even over macroevolutionary timescales, or that the relationship is adaptive and has been maintained by natural selection. Such trait correlations have been employed in the analyses of plant defense syndromes, defined as the convergent evolution of suites of correlated defense traits associated with some aspect of plant or herbivore ecology (Agrawal & Fishbein 2006). For latex in milkweeds, latex amount, cysteine protease activity and trichome density show positive correlated evolution (Agrawal & Fishbein 2006, 2008). Trade-offs were not evident between latex and several other defense traits, although we did find some evidence for a trade-off with cardenolides in latex (but not in leaf tissue) (Agrawal & Fishbein 2006, 2008). Despite the correlated structure of plant defense evolution, it is possible to find related milkweeds that are high in one defense trait and remarkably low in others (Zalucki & Malcolm 1999). For the resin canals of *Bursera*, Becerra et al. (2001) have reported similar correlated evolution, with species that have highly pressurized exudation of terpenes (that is, squirt gun defense) tending to have highly volatile terpene mixtures; the terpenes of low-flow *Bursera* species are more complex, viscous, and less volatile.

### SUMMARY POINTS: LATEX AS A DEFENSE SYSTEM

Below we summarize our view of how latex works as an effective, coordinated, and economical defense system.

1. The average concentration of defense substance in latex is often much higher than that found in leaves (and measures of leaf chemistry often include residual contents of laticifers) (e.g., 50–1000 times higher concentrations of defense chemicals or proteins are observed in latex of milkweeds, lettuce, and papaya; Seiber et al. 1982, Sessa et al. 2000, Konno et al. 2004), and this is what herbivores typically encounter when trying to consume plants with latex. Some compounds “stored” in latex may be autotoxic to the plant. Remarkably diverse defensive chemicals, including toxins and antinutritive compounds from all major classes of secondary metabolites, and various types of defense proteins are found in latex (see section on Biochemistry and Mode of Action).
2. Latex is mobilized and transported to the site of damage immediately after the damage e.g., latex can travel more than 70 cm to the damaged points in *Cryptostegia grandiflora* (Buttery & Boatman 1976). Since the defense substance accumulates at the point of damage (**Figure 1a**), latex is similar to an inducible defense system, although it is preformed in the plant.
3. Points 1 and 2 above indicate that latex is an economical system to provide a concentrated defense when and where it is needed most, with relatively small total amount of defense substances deployed. Other plant defenses appear to be far less mobile, especially directly following damage. This economy may be indicative of why latex is advantageous and successful as a defense against herbivory, and is consistent with its highly convergent evolution and apparent role as a key innovation in the radiation of plants.
4. Given points 1 and 2 above, latex can be seen as primarily effective against tiny mandibulate insects (e.g., mortality of specialist caterpillars feeding on milkweeds are the highest in the earlier instars and especially high at the first bites after hatching (Zalucki et al. 2001a,b). Larger herbivores that feed on whole plants (or even entire leaves) should be much less affected because the accumulation of latex at the site of damage will be ineffective and the exposure to defensive chemistry will not be concentrated.

5. Because laticifers are only effective when they can transport defense substances under pressure, their function is easily lost when the canals are disrupted. Indeed, this is the most common method of counterdefense (that is, vein-cutting and trenching; **Figure 1**) and consumption by adapted herbivores. This strategy is highly convergent and has evolved in three orders of insects.
6. The clotting activity of latex is essential so that defenses are not lost or wasted, wounds are sealed, and plant pressure is maintained. In most latex, clotting occurs in a few minutes after the damage and exposure to air. Additionally, clotting of latex is involved in the stickiness that gums up insect mouthparts or whole bodies.

### FUTURE ISSUES

Although alternative hypotheses for nondefensive functions of latex have been discounted, there are still large gaps in our understanding. The ease with which latex exudation can be estimated, the rich chemistry of compounds within latex, and the spectacular diversity and natural history of these plant-insect interactions make them ideal to study the ecology, evolution, and mechanisms of plant defense. For the future, we propose resolution of the following outstanding questions:

1. What is the genetic basis of latex production? Although quantitative genetic variation in latex production has been reported for a couple of systems, we know essentially nothing about heritability, costs and benefits of latex, the number of genes involved, or the identity and molecular evolution of latex genes.
2. Given the diversity of phytochemicals and proteins in the latex of any given species, what are the redundant, complementary, or synergistic effects of these components? What is the level of specificity in elicitation and impact of latex on different guilds of herbivores?
3. Beyond the temperate-tropical comparison, in what ecological contexts is latex found? A comparative assessment of latex either within clades or communities would be helpful to address this question.
4. Does latex play any role in tritrophic interactions (e.g., does time spent vein-cutting or trenching expose herbivores to predators)? Are predators/parasitoids attracted to sites where fresh latex is exuding?
5. Are there novel antiherbivore defense chemicals or proteins in plant latex belonging to new classes or with novel modes of action? Can these be exploited in plant breeding or pest management?
6. In addition to defense against herbivores, latex likely plays an equally important role in protecting plants against microbial pathogens. What are the physical effects of latex on wound healing; how can microbial entrance to the plant be prevented; and what are the chemical effects of antimicrobial compounds in latex?
7. When did latex evolve in different plant lineages, and likewise, when did vein-cutting and trenching evolve? Time-calibrated phylogenies should begin to allow us to answer these questions, and may also address the original functions of latex.

8. What, if any, adaptive role does latex play in species where it is exuded in only trace amounts (e.g., *Acer platanoides* and *Asclepias tuberosa*)?

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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