



Review

Plant latex and other exudates as plant defense systems: Roles of various defense chemicals and proteins contained therein

Kotaro Konno*

National Institute of Agrobiological Sciences, 1-2 Ohwashi, Tsukuba, Ibaraki 305-8634, Japan

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ABSTRACT

Plant latex and other exudates are saps that are exuded from the points of plant damage caused either mechanically or by insect herbivory. Although many (ca. 10%) of plant species exude latex or exudates, and although the defensive roles of plant latex against herbivorous insects have long been suggested by several studies, the detailed roles and functions of various latex ingredients, proteins and chemicals, in anti-herbivore plant defenses have not been well documented despite the wide occurrence of latex in the plant kingdom. Recently, however, substantial progress has been made. Several latex proteins, including cysteine proteases and chitin-related proteins, have been shown to play important defensive roles against insect herbivory. In the mulberry (*Morus* spp.)–silkworm (*Bombyx mori*) interaction, an old and well-known model system of plant–insect interaction, plant latex and its ingredients – sugar-mimic alkaloids and defense protein MLX56 – are found to play key roles. Complicated molecular interactions between Apocynaceae species and its specialist herbivores, in which cardenolides and defense proteins in latex play key roles, are becoming more and more evident. Emerging observations suggested that plant latex, analogous to animal venom, is a treasury of useful defense proteins and chemicals that has evolved through interspecific interactions. On the other hand, specialist herbivores developed sophisticated adaptations, either molecular, physiological, or behavioral, against latex-borne defenses. The existence of various adaptations in specialist herbivores itself is evidence that latex and its ingredients function as defenses at least against generalists. Here, we review molecular and structural mechanisms, ecological roles, and evolutionary aspects of plant latex as a general defense against insect herbivory and we discuss, from recent studies, the unique characteristics of latex-borne defense systems as transport systems of defense substances are discussed based on recent studies.

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* Tel.: +81 29 838 6087; fax: +81 29 838 6028.

E-mail address: konno@affrc.go.jp

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1. Introduction

Plant latex is a sap, typically a white sap, that is stored in the tissue called laticifer and that is exuded from a point of damage in plant tissues immediately after insect herbivory (see Figs. 1A, 2A, and 6A). More than 20,000 species from over 40 families of angiosperm plants exude latex (Lewinsohn, 1991), which is 8.9% of all angiosperm plants. The figure increases to 35,000 species when conifers and resin-exuding plants are included (Farrell et al., 1991). Latex contains a variety of chemicals and proteins, such as various terpenoids, alkaloids, rubber, and cardenolides as well as various proteins and enzymes such as proteases, chitinases, and glucosidases (see Section 4). In regard to latex's role, there have been several hypotheses, such as excretion of waste metabolites, coverage of damaged tissue, defense against herbivores, and defense against pathogens. Among these hypotheses, there is a lot of evidence to support the defensive roles against herbivores and pathogens, especially herbivores (Farrell et al., 1991). The earliest experimental observation was by Kniep, a German scientist, in the early 20th century (Kniep, 1905). He observed that Euphorbiaceae plant individuals whose leaves had been artificially damaged and that had no more exuded latex were damaged by slugs in outdoor conditions, whereas intact individuals with latex were not damaged (Kniep, 1905). More than a half century later, Dussourd and Eisner found that several specialist insects feeding on milkweeds have developed a vein-cutting behavior that can inactivate laticifer and stop the exudation of latex (Dussourd and Eisner, 1987). They also observed that the mandibles of beetles (*Tetraopes* spp.)

were trapped and glued by latex when milkweed latex was artificially placed on the mandibles (Dussourd and Eisner, 1987). Also, under natural feeding conditions, the mandibles of caterpillars that attempted to eat leaves, or the whole bodies of aphids that walked on plant surfaces became trapped by the latex of *Lactuca* species (Asteraceae) (Dussourd, 1993, 1995). Further, a large percentage of newly hatched monarch butterfly larvae (*Danaus plexippus*) were found trapped by milkweed latex (Zalucki and Brower, 1992; Zalucki et al., 2001a,b). These results suggested that plant latex, which is often sticky, defends plants against herbivorous insects by trapping and immobilizing them. On the other hand, some chemical ingredients in latex, such as morphine, an alkaloid, from poppy latex and cardenolides from milkweed latex, show apparent toxicity against animals, including insects. In these cases, such toxic chemicals are suggested to have defensive roles (Farrell et al., 1991). However, the roles of most of the remaining latex ingredients, especially various latex proteins, remain unknown. Also, not all latex and/or exudates are sticky enough to trap insects, such as the latex of mulberry trees, *Morus* spp. (Konno, unpublished data). Recently, a couple of findings have shown that various latex ingredients, notably latex proteins, play key defensive roles against insect herbivory (Konno et al., 2004, 2006; Wasano et al., 2009; Ramos et al., 2007, 2010). In this review, I present emerging observations that suggest the importance of latex ingredients – chemicals and proteins – in plant–insect interactions. Then, based on these observations, I discuss various aspects and characteristics of latex-borne defense and other canalicular defenses.

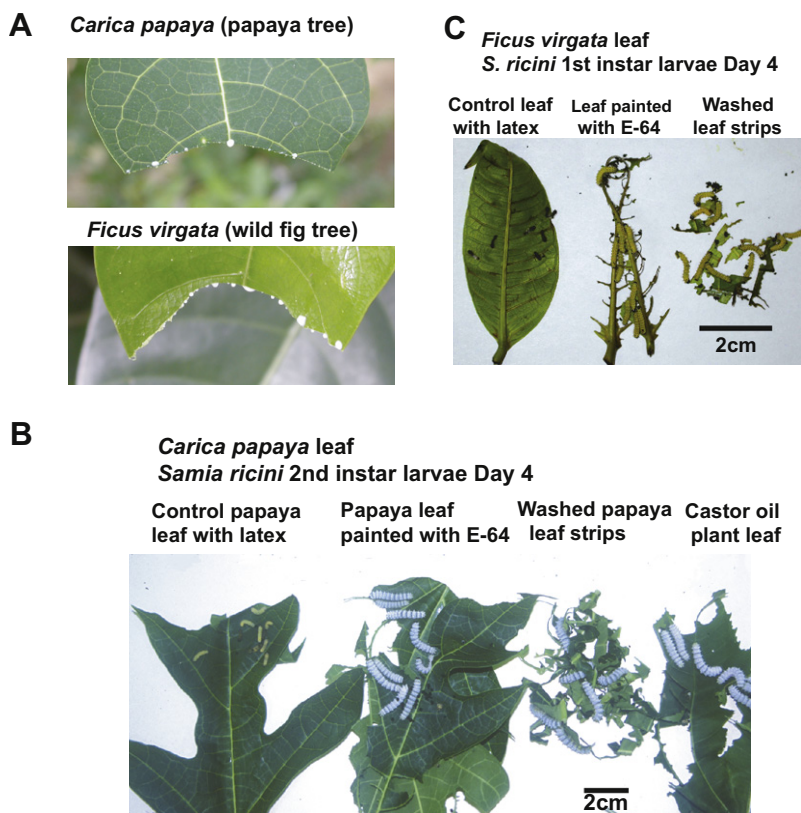


Fig. 1. Defensive activities of cysteine proteases in the latex of the papaya tree, *Carica papaya* (Caricaceae), and in that of the fig tree, *F. virgata*, against caterpillars. (A) Leaves of papaya (top) and fig trees (bottom) exuding latex rich in cysteine-protease activity from damaged veins. (B) Defensive activity of latex of the papaya tree and papain in it. Second instar Eri silkworm, *Samia ricini*, fed intact leaves containing latex, did not grow and died (left). However, leaf toxicity was completely lost after painting E-64, a cysteine protease-specific inhibitor, on the leaf surface (second from the left) or after removing the latex by washing out the leaf strips (third from the left), and the larvae grew as well as when they were fed leaves of their natural host, the castor oil plant, *Ricinus communis* (right). (C) Defensive activity of latex of a wild fig tree and the cysteine protease in it. Leaves of the wild fig tree show strong toxicity to the neonate Eri silkworm (left), but the toxicity was lost when E-64 was painted on leaves (center) or when the latex was washed off (right). The photo was taken on day 4 (modified from Konno et al., 2004).

2. General features and description of latex, resin, and other exudates

2.1. Anatomy of exuding canals: laticifers, resin ducts, oil ducts, and exuding phloem

By definition, latex is an exudate that is kept in the extremely elongated cells that extend along plant, and this canal structure that exudes latex is called laticifer. Because large vacuoles occupy a large part of laticifer cells and develop during laticifer formation (Cai et al., 2009), the majority of exuded latex is likely to originate in vacuoles. But the exuded latex may also contain fluid from the cytoplasm of laticifer cells in some cases, because in some laticiferous plants, the cytoplasm of laticifer cells degrades and is mixed with vacuoles in the final stage of laticifer development (Zhou and Liu, 2010). Latex typically is a non-transparent white sap (Figs. 1A and 2A), but in some cases it is clear and/or has a distinct color. Laticifers are distributed in roots, stems, petioles and leaves, and in leaves laticifers are associated mostly with leaf veins. Resin also is a sap exuded from damage. In some species, resin is transparent, but in other species resin is white color that resembles latex. Resin is distinct from latex in that it is kept in canalicular inter-cellular spaces called resin ducts and is not kept inside cells. If the fluid that is kept in the resin duct is oil (hydrophobic fluid), the duct is called an oil duct. Many plant species belonging to Cucurbitaceae exude ample saps from phloem. Laticifers are further classified into two sub-groups; nonarticulated laticifers and articulated laticifers (Dussourd and Denno, 1991; Hagel et al., 2008).

Nonarticulated laticifers (Fig. 5A) are formed from a small number of initial cells in the embryonic stage that elongate and often branch without cell division (but with the division of nuclei) to form a laticifer with huge multicellular tubular cells; species of Caricaceae and Moraceae typically have this type of laticifer (Dussourd and Denno, 1991; Hagel et al., 2008). Since the laticifers branch, but never merge, the formed laticifers have tree-like shapes without loop structures (Fig. 5A). Articulated laticifers (Fig. 5B) are formed from longitudinal chain of cells. Later the cell walls separating individual cells disappear and a tube-like structure is finally formed (Dussourd and Denno, 1991; Hagel et al., 2008). In some species (Caricaceae, Asteraceae), both longitudinal and lateral merges occur, resulting in net-like structures with loops (anastomosing laticifers) (Fig. 5B), but in other species (e.g., Convolvulaceae) only longitudinal merges occur resulting in a linear structure without loops (non-anastomosing laticifers) (Dussourd and Denno, 1991; Hagel et al., 2008).

2.2. Phylogenetical distribution

More than 35,000 species of vascular plants exude latex, resin and exudates, and over 20,000 species of flowering plants in over 40 families exudes latex. The families well known to contain large numbers of laticiferous plants are very widely scattered among many distant angiosperm lineages according APG systems (APG I, II, III), a classification system based on DNA sequence similarity (The Angiosperm Phylogeny Group, 1998, 2003, 2009). Representative laticiferous families include Papaveraceae (poppy: Eudicot:

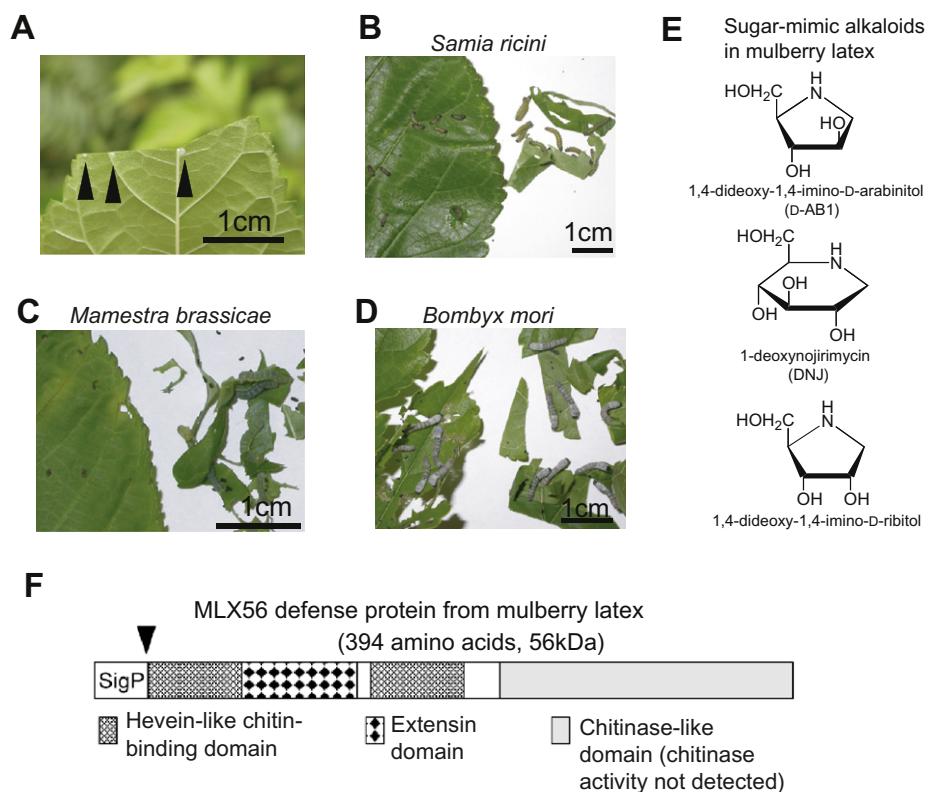


Fig. 2. Defense activity of mulberry latex against herbivorous insects and its defense substances. (A) Mulberry leaves exude latex from damaged veins (arrows). (B and C) Mulberry leaves with latex (left) shows toxicity against a generalist caterpillar species, the first instar larvae of Eri silkworm, *Samia ricini* (B), and the second instar larvae of the cabbage moth, *Mamestra brassicae* (C), but washed leaf strips of mulberry leaves without latex lose toxicity (right) and both *S. ricini* and *M. brassicae* grew well. (D) The first instar larvae of the silkworm, *Bombyx mori*, a mulberry specialist were not at all affected by mulberry latex and grew well on leaves with (left) or without (right) latex. Photos (B–D) were taken on day 4. (E) The three toxic sugar-mimic alkaloids (alkaloidal glycosidase inhibitors) that exist in the latex of mulberry trees (*Morus* spp.). In a wild *M. australis* population on Ishigaki Island, Okinawa, Japan, the concentrations of D-AB1, DNJ, and 1,4-dihydroxy-1,4-imino-D-ribitol reached 1.63%, 0.36%, and 0.48%, respectively. (F) The structure of MLX56 defense protein found in mulberry latex with two hevein domains and an extensin domain with chitin-binding activity. MLX56 showed toxicity to the generalist larvae of *S. ricini* and *M. brassicae* at very low concentrations (0.01–0.03%), and was highly resistant to the digestion by proteases. Interestingly, MLX56 is not toxic to the silkworm, *B. mori*, a mulberry specialist (modified from Konno et al., 2006; Wasano et al., 2009).

Ranunculales), Moraceae (fig and mulberry: Core Eudicot: Rosid: Eurosid I (Fabid): Rosales), Caricaceae (papaya tree: Core Eudicot: Eurosid II (Malvid): Brassicales), Apocynaceae (milkweed: Core Eudicot: Euasterid I (Lamiid): Gentianales), Convolvulaceae (sweet potato: Core Eudicot: Euasterid I (Lamiid): Solanales), Asteraceae (lettuce and dandelion: Core Eudicot: Euasterid II (Campanulid): Asterales) and Campanulaceae (cardinal plant: Core Eudicot: Euasterid II (Campanulid): Asterales), Araceae (taro: Monocot: Alismatales), Alliaceae (onion: Monocot: Asparagales) and Musaceae (banana: Monocot: Commelinid: Zingiberales). Plants of Anacardiaceae (lacquer plant: Core Eudicot: Eurosid II (Malvid): Sapindales) exude a latex-like resin from resin ducts. Those of Apiaceae (parsley, parsnip: Core Eudicot: Euasterid II (Campanulid): Apiales) exude oil from oil ducts. Those of Cucurbitaceae (pumpkin and cucumber: Core Eudicot: Rosid: Eurosid I: Cucurbitales) emit phloem sap. It is interesting that most families with exuding species have closely related families in the same orders without exuding species (e.g., Moraceae vs. Rosaceae of Rosales; Caricaceae vs. Brassicaceae of Brassicales; Apocynaceae vs. Rubiaceae of Gentianales; Musaceae vs. Cannaceae of Zingiberales). These distribution patterns suggest that exudates such as latex and resin are highly convergent traits that have evolved several times independently, although there could have been some cases in which latex has been lost from certain lineages. Farrell et al. (1991) concluded that latex and resin have evolved at least 40 times from phylogenetic analyses according to Cronquist's morphology-based classification, but it is obvious that the phylogenetic analyses depending on APG classi-

fication based on DNA sequence similarity also support that the exudation of latex and resin is a widely distributed and highly convergent trait.

2.3. Geological distribution

As a general trait, latex is more frequently observed in tropical plants. For example, while plant families and species that prevail in tropical areas contains high percentages of laticiferous families and species (respectively, 12.2% for families and 14.0% for species), plant families and species prevailing in temperate areas are less laticiferous (4.9% for families and 5.9% for species) (Lewinsohn, 1991). Regional surveys showed that around 15–30% of plant species exude latex in tropical Africa (Reitsma, 1988), and 20–35% of those exude latex in Tropical America (Amazon) (Lewinsohn, 1991) compared to 8.9% worldwide (Farrell et al., 1991). Since the interactions between plant and herbivorous insects are more intense in tropical regions than in temperate regions, the frequent occurrence of laticiferous plants is consistent with the defensive roles of latex and laticifer against herbivorous insects.

2.4. Physical characteristics of latex: color, exudation, stickiness, and clotting

Latex is typically a non-transparent white sap such as in poppy (*Papaver somniferum*, Papaveraceae), milkweed (*Asclepias syriaca*, Apocynaceae), fig tree (*Ficus carica*, Moraceae) and rubber tree

(*Hevea brasiliensis*, Euphorbiaceae), but some plant latex is transparent and/or has distinct colors even in species closely related to those that have typical non-transparent white latex, such ascelandine (*Chelidonium majus*, Papaveraceae, yellow–orange transparent), oleander (*Nerium indicum*, Apocynaceae, transparent without color), wild fig trees (*Ficus septica*, pale yellow non-transparent, *Ficus benguetensis*, orange non-transparent, Moraceae). In some plant species, latex is highly viscous that can mire the whole body or the mouth part of an insect. Species that produce such latex include milkweed, *A. syriaca*, rubber tree, *H. brasiliensis* and lettuce, *Lactuca sativa* (Dussourd and Eisner, 1987; Dussourd, 1995), whereas the latex of others species is not sticky, such as the latex exuded from leaves of the mulberry tree, *Morus* spp., and the oleander tree, *N. indicum*. In most cases, latex becomes stickier after it is exuded, and in some cases it finally clots. The amounts of latex exuded from a point of damage differ significantly even among closely related species within the same genus and family. For example, young leaves of *Asclepias barjonifolia* exude four times more latex per equal size of wound than leaves of *Asclepias angustifolia* (Rasmann et al., 2009). Similarly, while *Ficus variegata* and *Ficus virgata* exude large amounts of white non-transparent latex, *F. benguetensis* exudes a very small amount of orange-colored non-transparent latex (Konno et al., unpublished data).

2.5. Existence of various chemicals and proteins

Latex, resin, and other exudates contain a great diversity of secondary metabolites and proteins, especially defense chemicals and proteins, in a species-specific manner. A variety of secondary metabolites such as alkaloids, terpenoids, cardenolides, rubber, phenolics, furanocoumarins, and starch, as well as a variety of proteins such as proteases, oxidases, lectins, chitin-binding proteins, chitinases, glucosidase, and phosphatase exist in latex, resin, and exudates in highly concentrated manners (Table 1). Until recently, the roles of these chemicals and proteins were unknown in most cases, but recent discoveries (see Section 3) suggest that many of them have roles in plant defense against herbivore insects. The details of various ingredients of latex, resin and exudates and their identified and potential roles in plant defense against herbivores are discussed in Section 4.

3. Examples of defensive roles of plant latex and its ingredients against herbivorous insects

3.1. Emerging examples from systems where the defensive roles of latex were newly found

3.1.1. Defensive roles of cysteine proteases and other defense proteins in latex

A variety of proteins and enzymes have been found from plant latex. Cysteine proteases, serine proteases, chitinases, lectins, and oxidases are examples (Table 1, see Section 4.2 for detail). Among them, cysteine proteases such as papain from the latex of the papaya tree, *Carica papaya* (Fig. 1A, top), and ficin from the latex of fig trees (*F. carica*, *Ficus* spp.) (Fig. 1A, bottom) have been well known for a long time and have been well characterized (Kimmel and Smith, 1954; Sgarbieri et al., 1964; Kramer and Whitaker, 1964; Cohen et al., 1986). Papaya latex (Fig. 1A) containing a high titer of papain has been collected in large amounts and papain has been used as a meat tenderizer in the food industries. The reason for existence of papain for the papaya tree, however, has never been explained. Recently, papaya leaves were found to have a strong lethal toxicity to the larvae of generalist caterpillars, such as the Eri silkworm, *Samia ricini* (Fig. 1B, left), and the cabbage moth, *Mamestra brassicae*, and showed strong growth retardation

in the armyworm, *Spodoptera litura* (Konno et al., 2004). Interestingly, the strong toxicity was gone and larvae grew very well when the leaves were cut and the latex was washed (Fig. 1B, third to the left), or when the leaves were painted with E-64, a cysteine protease-specific inhibitor (Fig. 1B, second to the left), and they grew as well as when they were fed the castor oil plants, their natural host (Fig. 1B, right) (Konno et al., 2004). The same results were obtained using the leaves of wild fig, *F. virgata* (Fig. 1C) (Konno et al., 2004). These results indicate that cysteine protease in papaya and fig latex has a crucial defensive role against Lepidopteran herbivores. The cysteine protease activities were highly concentrated in latex. The titer of cysteine protease is 200–500 times higher in latex than in the whole leaf on average, and is 20 times higher than the lethal dose (Konno et al., 2004). The cysteine proteases, papain, ficin, and bromelain from pineapple all showed toxicity to larvae, indicating the general toxicity of cysteine proteases to insects (Konno et al., 2004). This was the first demonstration of latex protein having a defensive role against herbivores. Proteases are widely found in latex from a variety of plants such as cysteine proteases from the latex of Caricaceae, Moraceae, and Apocynaceae (Kimmel and Smith, 1954; Sgarbieri et al., 1964; Arribère et al., 1998); serine proteases from Moraceae, Euphorbiaceae, Apocynaceae, Convolvulaceae (Singh et al., 2008; Arima et al., 2000; Tomar et al., 2008; Patel et al., 2007); and both cysteine and serine proteases from the latex-like resin of mango (Saby et al., 2003). It is possible that these proteases may all function as a defense against insects.

Further, there direct observation has accumulated to indicate the defensive roles of latex proteins against insects. First, the protein fraction of latex of *Calotropis procera* (Apocynaceae) where all small molecules were dialyzed showed apparent toxicities against the larvae of *Anticarsia gemmatilis* (Lepidoptera: Noctuidae) (Ramos et al., 2007) and the bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae) when mixed into an artificial diet at a 0.1% concentration (Ramos et al., 2010). Although these studies on *Calotropis* latex did not identify which protein was responsible for its strong toxicity, the latex exhibited protease inhibitor, chitinase, and cysteine protease activities, all of which are regarded as defense-related proteins and candidates for the protein responsible for defensive activity of the latex (Ramos et al., 2007, 2010). Secondly, a Romaine lettuce variety that is resistant to attack by the banded cucumber beetle, *Diabrotica balteata* (Coleoptera: Chrysomelidae), shows the induction of several latex proteins, polyphenol oxidase (PPO), peroxidases (POD), and phenylalanine ammonia lyase (PAL) (Sethi et al., 2009). It is not clear whether these proteins function as defenses directly against insects, by activating the precursor molecules of defense chemicals, or by synthesizing defense chemicals. However, there is evidence that PPO and POX act as defense proteins by activating phenolics into active molecular species (e.g., quinones) that destroy nucleophilic amino acids such as cysteine and lysine and thereby decreasing the nutritive values of dietary proteins (Felton and Gatehouse, 1996). Third, a novel chitin-binding protein, MLX56 (Fig. 2F), was purified from mulberry latex and showed strong inhibition against generalist lepidopteran herbivores at very low concentrations (0.01%) (Wasano et al., 2009). We described this in detail in the next section. Increasing numbers of observations on the existence of defense proteins in latex indicate that considerable parts of various latex proteins may have defensive roles against herbivores. In other words, latex could be a promising source of defense proteins that need to be studied.

3.1.2. The mulberry–silkworm system

The relationship between mulberry, *Morus* spp., and the silkworm, *Bombyx mori*, is one of the oldest and best-recognized plant–insect interactions, due to the long history of sericulture. There have been some attempts to address the molecular bases of the mulberry–silkworm interactions; attempts have been made

Table 1

Chemicals and proteins found in plant latex that have confirmed or potential defense role against herbivorous insects.

Category	Compounds and Proteins	Plant species and references
Chemicals		
Alkaloids	Morphine , <i>Papaver somniferum</i> (Papaveraceae) (Itenov et al., 1999; Hartmann, 1991); Cheledonine , Sanguinarine , Copticine , <i>Chelidonium majus</i> (Papaveraceae) (Tomè and Colombo, 1995); Lobeline , <i>Lobelia cardinalis</i> (Campanulaceae) (Oppel et al., 2009); Sugar-mimic alkaloids , D-AB1 , DNJ , etc. <i>Morus australis</i> , <i>Morus</i> spp. (Moraceae) (Konno et al., 2006); Phenanthroindolizidin alkaloids , <i>Ficus</i> spp., (Konno et al., unpublished)	
Terpenoids	Lactucin , Lactucopicrin , Lettucenin A , <i>Lactuca</i> spp., <i>Lactuca sativa</i> (Asteraceae) (Sessa et al., 2000; Rees and Harborne, 1985; Dussourd, 2003); Phorbol , <i>Euphorbia</i> spp., <i>Euphorbia biglandulosa</i> (Noack et al., 1980; Gershenzon and Croteau, 1991).	
Cardenolide	Voruscharin , Ushcharidin , Usharin , Calotropagenin etc. <i>Asclepias</i> spp., <i>Asclapias curassavica</i> , etc. <i>Calotropis procera</i> (Apocynaceae) (Seiber et al., 1982; Malcolm, 1991; Dussourd and Hoyle, 2000; Rasmann et al., 2009); Toxicariosides , <i>Antiaris toxicaria</i> (Moraceae) (Carter et al., 1997)	
Rubber	Rubber (cis-1,4-isoprene polymer) , <i>Hevea brasiliensis</i> (Euphorbiaceae), <i>Ficus</i> spp. (Moraceae), <i>Alstoeia boonei</i> (Apocynaceae), <i>Parthenium argentatum</i> , <i>Lactuca</i> spp. (Asteraceae) (Mooibroek and Cornish, 2000; Bushman et al., 2006)	
Phenolics	p-Coumaric acid hexadecyl, octadecyl, eicosyl esters , <i>Ipomoea batatas</i> (Convolvulaceae) (Snook et al., 1994); Urushiol , <i>Rhus</i> (<i>Toxicodendron</i>) spp. (Anacardiaceae, Resin) (Dawson, 1954)	
Furanocoumarins	Bergapten , Xanthotoxin , Angelicin , <i>Petroselinum crispum</i> , <i>Pastinica sativa</i> (Apiaceae, resin oil) (Berenbaum 1991; Wu and Hahlbrock, 1992; Reinold and Hahlbrock, 1997; Wen et al., 2006; Chambers et al., 2007)	
Proteins		
Proteases	Cysteine protease , <i>Carica papaya</i> (Caricaceae), <i>Ficus carica</i> (Moraceae), <i>Morrenia brachystephana</i> , <i>Calotropis procera</i> , <i>Asclepias barjonifolia</i> (Apocynaceae), <i>Mangifera indica</i> (Anacardiaceae, resin) (Kimmel and Smith, 1954; Konno et al., 2004; Sgarbieri et al., 1964; Arribère et al., 1998; Ramos et al., 2010; Rasmann et al., 2009; Saby et al., 2003) Serine protease , <i>Ficus elastica</i> (Moraceae), <i>Hevea brasiliensis</i> , <i>Euphorbia sapina</i> (Euphorbiaceae), <i>Wrightia tinctoria</i> (Apocynaceae), <i>Ipomoea carnea</i> (Convolvulaceae), <i>Mangifera indica</i> (Anacardiaceae, resin) (Lynn and Clevette-Radford, 1986a,b; Arima et al., 2000; Tomar et al., 2008; Patel et al., 2007; Saby et al., 2003)	
Protease inhibitors	Cysteine protease inhibitor , <i>Calotropis procera</i> (Apocynaceae), <i>Cucurbita maxima</i> (Cucurbitaceae, phloem sap) (Ramos et al., 2010; Walz et al., 2004; Kehr, 2006) Serine protease inhibitor (Trypsin inhibitor and chymotrypsin inhibitor) , <i>Ficus carica</i> (Moraceae), <i>Carica papaya</i> (Caricaceae), <i>Hevea brasiliensis</i> (Euphorbiaceae), <i>Cucurbita maxima</i> (Cucurbitaceae) (Kim et al., 2003; Azarkan et al., 2004; Walz et al., 2004; Kehr, 2006) Aspartic protease inhibitor , <i>Cucurbita maxima</i> (Cucurbitaceae) (Walz et al., 2004; Kehr, 2006)	
Oxidase	Polyphenol oxidase (PPO) , <i>Hevea brasiliensis</i> (Euphorbiaceae), <i>Taraxacum kok-saghyz</i> , <i>Lactuca sativa</i> (Asteraceae), <i>Mangifera indica</i> (Anacardiaceae, Resin) (Wititsuwannakul et al., 2002; Wahler et al., 2009; Sethi et al., 2009) Peroxidase (POD) , <i>Ficus carica</i> (Moraceae), <i>Ipomoea carnea</i> (Convolvulaceae), <i>Lactuca sativa</i> (Asteraceae), <i>Mangifera indica</i> (Anacardiaceae, Resin) (Kim et al., 2003; Patel et al., 2008; Sethi et al., 2009; Saby et al., 2003) Lipoxygenase (LOX) , <i>Cucurbita maxima</i> (Cucurbitaceae, phloem sap) (Walz et al., 2004)	
Lectins, Chitin-binding proteins, and Chitinases	Lectin (inhibited by lactose and D-galactose) , <i>Euphorbia lactea</i> , <i>Euphorbia hermentiana</i> , etc. (Euphorbiaceae) (Lynn and Clevette-Radford, 1986c) Chitin-binding protein , (Hevein-like) <i>Hevea brasiliensis</i> (Euphorbiaceae), <i>Morus alba</i> (Moraceae) (Gidrol et al., 1994; Broekaert et al., 1990; Wasano et al., 2009) GlcNAc-binding (Chitin-binding) protein (non-hevein like) , <i>Cucurbita maxima</i> (Cucurbitaceae, phloem sap) (Read and Northcote, 1983; Walz et al., 2004; Kehr, 2006; Van Damme et al., 1998) Chitinase (also chitin-binding) , <i>Calotropis procera</i> (Apocynaceae), <i>Morus alba</i> (Moraceae) (Ramos et al., 2010; Kitajima et al., 2010)	
Others	Lipase , <i>Euphorbia characias</i> (Euphorbiaceae), <i>Asclepias curassavica</i> (Apocynaceae), <i>Carica papaya</i> (Caricaceae) (Giordani et al., 1991; Fiorillo et al., 2007; Gandhi and Mukherjee, 2000) Glutamyl cyclase , <i>Carica papaya</i> (Caricaceae) (Azarkan et al., 2004) Gum arabic glycoprotein , <i>Acacia senegal</i> (Fabaceae) (Goodrum et al., 2000) Phenyl alanine ammonia lyase (PAL) , <i>Lactuca sativa</i> (Asteraceae) (Sethi et al., 2009) Phosphatase , <i>Euphorbia esula</i> , <i>Euphorbia splendens</i> (Euphorbiaceae) (Lynn and Clevette-Radford, 1987b) Linamarase (β-glucosidase) , <i>Manihot esculenta</i> (Euphorbiaceae) (Nambisan, 1999)	

in term of feeding attractants (Hamamura, 1959). However, our recent study showed that there is a “plant defense – insect adaptation” type of relationship between mulberry tree and silkworm, where mulberry latex and its defensive ingredients play crucial roles (Konno et al., 2006; Hirayama et al., 2007; Daimon et al., 2008; Wasano et al., 2009). We found that mulberry leaves are toxic to several lepidopteran larvae such as the oligophagous larvae of the Eri silkworm, *S. ricini* (Saturniidae), and the polyphagous larvae of the cabbage moth, *M. brassicae* (Noctuidae) (Konno et al.,

2006). When mulberry leaves were given to these generalist larvae, the larvae bit into the leaves but did not grow and eventually died (Fig. 2B and C left). However, when the leaves were cut and washed in order to remove latex, the leaves lost toxicity and larvae grew normally (Fig. 2B and C left) (Konno et al., 2006). Interestingly, the silkworm, *B. mori*, a mulberry specialist, was not at all affected by the existence of latex, and grew equally well on leaves with or without it (Fig. 2D), suggesting that *B. mori* has developed some adaptive mechanism against mulberry latex. This is discussed later

in this section. Mulberry leaves exude latex (Fig. 2A). When caterpillars bite the leaves, large amounts of latex relative to the tiny larvae appear in front of the caterpillar (cf. Fig. 6A), and the larvae seem to be forced to drink large amounts of latex while feeding on mulberry leaves. Mulberry latex itself shows toxicity to generalist caterpillars, such as the larvae of Eri silkworm, *S. ricini*, and the cabbage moth, *M. brassicae*, when added to an artificial diet. Further purification showed that there are two types of factors in latex responsible for defense (Fig. 2E and F). The first type is a series of sugar-mimic alkaloids contained in latex (Fig. 2E) (Konno et al., 2006). For example, the latex of mulberry tree, *Morus australis*, native to Okinawa, Japan contain three different sugar-mimic alkaloids: 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1, 1.63% wet latex), 1-deoxynojirimycin (DNJ, 0.36%), and 1,4-dideoxy-1,4-imino-D-ribitol (0.48%) (Fig. 2E) (Konno et al., 2006). Sugar-mimic alkaloids, which are also called imino sugars or polyhydroxy alkaloids, are alkaloids whose structures resemble those of sugar molecules and are known to function as strong inhibitors of various human and insect glycosidases at very low concentrations (10^{-4} – 10^{-6} M). These alkaloids are suggested to have defensive roles against herbivorous insects (Asano et al., 2000; Hartmann, 1991). However, the concentrations of the three sugar-mimic alkaloids in the latex are much higher than these concentrations, reaching 2.5% altogether (more than 150 mM) in the latex of *M. australis* (Konno et al., 2006). Also, this concentration is much higher (ca. 100 times) than previously reported concentrations of sugar-mimic alkaloids from mulberry leaves (0.01–0.1%). Since it is likely that young larvae will confront a large amount of latex relative to their small body size (Fig. 6A), the actual concentration of sugar-mimic alkaloids that the larvae uptake will be close to the concentration of sugar-mimic alkaloids in mulberry latex. Bioassays with artificial diets showed 0.04–0.1% (0.003–0.007 M) of D-AB1 or DNJ significantly inhibits the growth of *S. ricini* larvae, which do not normally feed on mulberry leaves (Konno et al., 2006; Hirayama et al., 2007). This result suggests the sugar-mimic alkaloids in mulberry latex function as a defense against nonadapted herbivores. The toxicity of sugar-mimic alkaloids against insects is exerted by the inhibition of at least two glycosidase activities, sucrose and trehalase activities (Hirayama et al., 2007). Sucrose is the major sugar source for insects that feed on mulberry leaves. Sucrose is degraded into glucose and fructose by sucrose activity and absorbed in the hemolymph, and then is synthesized into trehalose, which exists in the hemolymph as the reservoir of sugar. When necessary, trehalose is degraded into glucose by trehalase and consumed as an energy source in each tissue (Fig. 3A). In the generalist *S. ricini*, sugar-mimic alkaloids inhibited absorption of sugar supplied to the diet in the form of sucrose, but did not inhibit the absorption sugar supplied in the form of glucose (Hirayama et al., 2007). The sucrose activity in the midgut tissue of *S. ricini* is inhibited by 1–10 μ M of D-AB1 or DNJ (Fig. 3B and C) (Hirayama et al., 2007). These data indicate that sugar-mimic alkaloids inhibit the absorption of sucrose by inhibiting sucrose activity in the midgut of *S. ricini*. However, sucrose was not the only target of the toxicity of sugar-mimic alkaloids. These alkaloids also disturb trehalose metabolism (Fig. 3A). When *S. ricini* were fed diets containing sugar-mimic alkaloids, the trehalose concentration in the hemolymph rose significantly (Hirayama et al., 2007). Sugar-mimic alkaloids existed in the hemolymph of *S. ricini* in concentrations high enough to inhibit trehalase activities in various tissues (Hirayama et al., 2007). These observations suggested that the sugar-mimic alkaloids exert toxicity also by inhibiting the utilization of trehalose, the blood sugar, by inhibiting trehalase in various tissues in *S. ricini*; as a consequence, the concentration of unutilized trehalose rises. In generalists, both sugar metabolisms, digestion and absorption of sucrose (the major sugar source), and the utilization of trehalose (the major sugar reservoir as blood sugar), are inhibited,

resulting in severe toxicity. Interestingly, the sugar-mimic alkaloids found in mulberry latex showed no toxicity against the silkworm, *B. mori*, a mulberry specialist (Konno et al., 2006). Also, *B. mori* shows no behavioral adaptations, such as vein cutting and trenching, which will be discussed later in this review. As suggested from these observations, *B. mori* larvae have developed a physiological adaptation to sugar-mimic alkaloid. In *B. mori*, neither sucrose absorption nor trehalose concentration in the hemolymph was affected when sugar-mimic alkaloids were added to the diet (Hirayama et al., 2007). Further, neither sucrose nor trehalase activity in various tissues was inhibited by sugar-mimic alkaloids *in vitro* (Hirayama et al., 2007). While the sucrose activity of the generalist *S. ricini* was inhibited by 1–10 μ M of DNJ or D-AB1, the sucrose activity of *B. mori* was not inhibited by 1000 μ M of sugar-mimic alkaloids (Fig. 3B and C) (Hirayama et al., 2007). Similarly, the trehalase activities of *B. mori* were much less sensitive than those of *S. ricini* (IC₅₀ is 10–50 times higher in *B. mori*) (Hirayama et al., 2007). These results indicated that the silkworm, *B. mori*, has evolved glycosidases that are insensitive to sugar-mimic alkaloids in mulberry latex. Further, the molecular mechanisms underlying the enzymatic adaptation of *B. mori* to sugar-mimic alkaloids have been clarified by recent studies enhanced by the genome project of *B. mori* (Daimon et al., 2008). It has been known that sucrose activities are represented by two different types of enzymes: α -glucopyranosidases, which recognize glucose moiety of sucrose as its substrate and which are inhibited by sugar-mimic alkaloids, and β -fructofuranosidases, which recognize the fructose moiety of sucrose but are not inhibited by sugar-mimic alkaloids. Most insects, including the generalist *S. ricini*, adopt an α -glucopyranosidase-type sucrose as a digestive enzyme in the midgut. However, in a mulberry specialist *B. mori*, sugar-mimic alkaloid-insensitive β -fructofuranosidase-type sucrose, which had been moved into the silkworm genome by horizontal transmission from the bacterial genome, was adopted as a digestive enzyme (Daimon et al., 2008). Since the β -fructofuranosidase type of sucrose genes is widely found among lepidopteran insects, the horizontal transmission from the bacterial genome may in itself have had nothing to do with adaptation to the hostplants at the beginning, and may have been retained in the lepidopteran genome for some unknown functions other than the digestion of sucrose in the midgut. Much later, when an ancestor of the silkworm, *B. mori*, made the host shift to the mulberry, the β -fructofuranosidase-type sucrose may have been adopted as the digestive sucrose in the midgut.

Sugar-mimic alkaloids are not the only defensive factor in mulberry latex. Another defense factor is equally important. We have recently purified a novel defense protein, MLX56, and have cloned its gene. MLX56 is a protein with 394 amino acids and has a structure with one extensin domain between two hevein-like chitin-binding domains (hevein domain) in the N-terminal regions, as well as an inactive chitinase-like domain in the C-terminal (Fig. 3C) (Wasano et al., 2009). As was expected from its hevein-like chitin-binding domains, MLX56 had strong chitin-binding activity. As was expected from its extensin domain, MLX56 was highly glycosylated. However, despite of the existence of the chitinase-like domain, chitinase activity was not detected. As is often the case with the plant defense proteins that function in the insect midgut (Chen et al., 2007), MLX56 is highly resistant to the digestive activities of digestive juices of lepidopteran larvae and of proteases such as trypsin and chymotrypsin, and stays intact after 24 h treatments (Wasano et al., 2009). MLX56 is highly toxic to lepidopteran insects, such as the generalist *S. ricini* and the polyphagous pest species *M. brassicae* at very low concentration (0.01–0.03% protein/wet-weight artificial diet), and its toxicity (growth inhibition) is one of the strongest among plant-derived defense protein (Wasano et al., 2009). Interestingly enough, again the silkworm, *B. mori*, is resistant to MLX56 as well as to sugar-mimic alkaloids

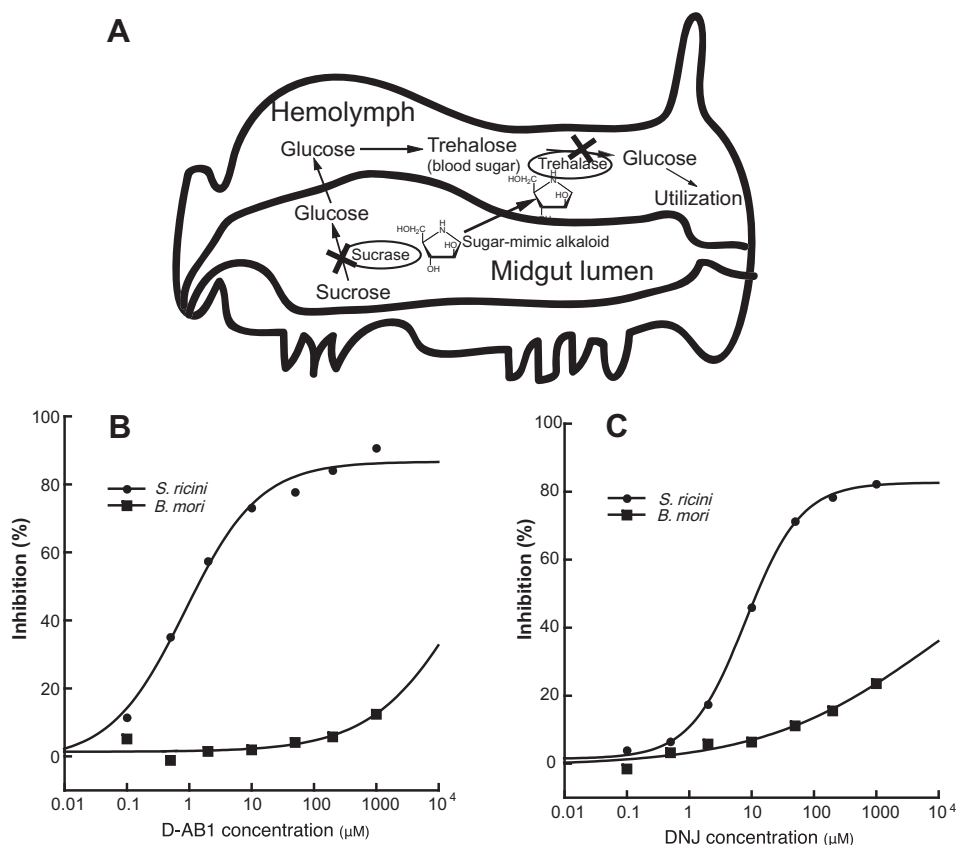


Fig. 3. Physiological adaptation of the silkworm, *Bombyx mori*, to the toxicity of sugar-mimic alkaloids. (A) Schematic model of toxic mechanisms of sugar-mimic alkaloids. When insects feed on plant leaves, sucrose, the major sugar source in foliage, is degraded into glucose by sucrase. Glucose then is absorbed into hemolymph and is then synthesized into trehalose, the blood sugar of insects. In each tissue, trehalose is degraded into glucose by trehalase. Sugar-mimic alkaloids exert toxicity by inhibiting both sucrase and trehalase activities, thus disturbing both the absorption and consumption of sugar in generalist insects not specialized in feeding on mulberry leaves. The silkworm, *B. mori*, can adapt to the toxicity of sugar-mimic alkaloids, because both sucrase and trehalase are insensitive to sugar-mimic alkaloids and because sugar absorption and consumption are not inhibited by sugar-mimic alkaloids. (B and C) Differential inhibitory effects of sugar-mimic alkaloids on midgut soluble sucrase activities of fourth-instar larvae of *S. ricini*, a generalist, and *B. mori*, a mulberry specialist. (B) Inhibitory effects of D-AB1. (C) Inhibitory effects of DNJ. The sucrase activity of *B. mori* was inhibited by much lower concentrations of sugar-mimic alkaloids than was the sucrase activity of *S. ricini* (modified from Hirayama et al., 2007).

(Wasano et al., 2009). Probably, *B. mori* has developed an unknown physiological adaptation to MLX56. Since MLX56 binds to chitin, a major component of insect cuticle and peritrophic membrane in the midgut lumen, the chitin of the insect is likely to be involved. The overall toxic mechanism of MLX56 is still unclear.

As described above, latex and its ingredients play key roles in mulberry–silkworm interaction, one of the oldest-known and best-studied plant–insect interactions. The evolution of sophisticated physiological adaptation in specialist herbivores against latex ingredients (in the present case, sugar-mimic alkaloids) is strong evidence that the latex ingredients play a crucial role in plant defense and have been imposing selective pressures on herbivorous insects. Since the silkworm, *B. mori*, is a well-known model insect, whose whole genome has recently been sequenced, much more about the role of latex in plant–insect interaction will be elucidated from the mulberry–silkworm system.

3.2. New evidence from systems where the defensive roles of latex have been known for a long time

3.2.1. Latex of milkweeds and Apocynaceae plants: cardenolides and defense proteins

The latex of milkweeds (i.e., *Asclepias* spp.) and related Apocynaceae plants is known to contain cardenolides (Fig. 4A) or cardiac glycosides, although the concentrations in latex differ among species (trace amount to 30% dry weight) (Malcolm, 1991; Seiber et al.,

1982). Cardenolides are inhibitors of Na^+/K^+ -ATPase. Since Na^+/K^+ -ATPase plays an important role in maintaining electric potential in most animal cells and is particularly important in maintaining nerve activities, cardenolides are highly toxic to animals. For their universal toxicity against animals, including insects, cardenolides in the latex of Apocynaceae have long been believed to function as a defense of these plants against herbivores (Malcolm, 1991). Probably, the cardenolide-containing latex of Apocynaceae plants and the specialists on it, such as the Monarch butterfly, are the first plant–herbivore systems from which we can infer that latex ingredients play an important role in plant defense against herbivorous insects. This inference is supported by the general existence of behavior to circumvent latex, such as vein-cutting and trenching, in the specialist feeders of Apocynaceae insects such as the adults of *Labidomera clivicollis* (Coleoptera) and the larvae of the queen butterfly, *Danaus gilippus* (Lepidoptera) feeding on milkweed, *A. syriaca* (Apocynaceae) (Dussourd and Eisner, 1987). Nevertheless, direct and detailed evidence that cardenolides function as a defense has been scarce. Recently, however, evidence has accumulated that shows cardenolides in Apocynaceae function as a plant defense against herbivorous insects. First, both the latex of *Asclepias curassavica* and the cardenolides contained in the latex caused toxic symptoms such as regurgitation, convulsions with spasms, and then immobilization and unresponsiveness lasting over a day in generalist caterpillars, *Trichoplusia ni* (Noctuidae), after the larvae ingested the latex or the cardenolides (Dussourd and Hoyle,

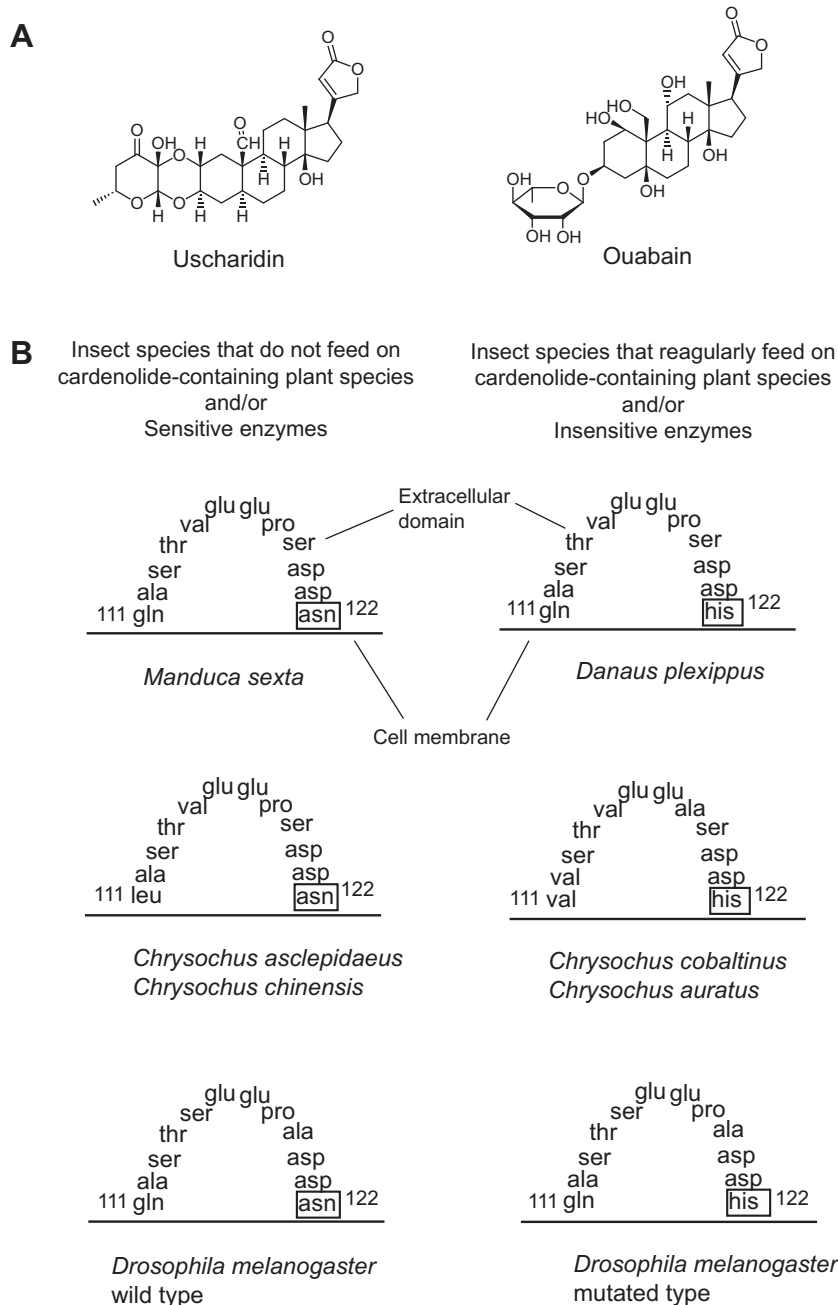


Fig. 4. Relationship between Apocynaceae plants that exude cardenolide-rich latex and specialist insects. (A) Example of cardenolides from Apocynaceae plants. Uscharidin (left) is a major latex cardenolide in milkweed species such as *Asclepias curassavica* and *A. vestita*, which contain very high concentrations of cardenolides. Uscharidin is also contained in the latex of *Calotropis procera*. Ouabain (right) is a well-studied cardenolide from the African plant *Acokanthera ouabaio*. (B) Physiological adaptation in Na^+/K^+ -ATPases of specialist insects feeding on Apocynaceae plants that exude latex containing cardenolides. Na^+/K^+ -ATPase is the target enzyme in cardenolide toxicity. The extramembrane parts of Na^+/K^+ -ATPase that include cardenolide binding sites (amino acid 122, boxed amino acids) are compared among insect species. The Na^+/K^+ -ATPases of insects that feed on plants exuding cardenolide-containing latex are often insensitive to cardenolides, such as the Na^+/K^+ -ATPase of monarch butterfly, *Danaus plexippus*, feeding on the milkweeds, *Asclepias syriaca* and *A. curassavica* as well as *Apocynum cannabinum*. The Na^+/K^+ -ATPases of insects feeding on cardenolide-containing Apocynaceae plants, such as *D. plexippus*, *Chrysochus cobaltinus* (Coleoptera), and *C. auratus* have histamine in position 122 of the cardenolide binding site (enzymes in the right column), while those of non-cardenolide-adapted species, *Manduca sexta* (Lepidoptera), *C. asclepiadaeus*, *C. chinensis*, and *Drosophila melanogaster* have asparagine in position 122 (enzymes in the left column). While the human embryonic kidney cell line, HEK (which is sensitive to ouabain), transformed by modified *Drosophila* Na^+/K^+ -ATPase whose Asn122 was substituted by His122 (right bottom) became tolerant to ouabain and survived, the HEK cells transformed by wild-type Na^+/K^+ -ATPase with Asn122 (left bottom) remained susceptible and died in ouabain-containing culture media (figure prepared based on the results of Holzinger and Wink, 1996; Labeyrie and Dobler, 2004).

2000). Other noctuid caterpillars, *Rachiplusia ou*, *Anagrapha falcifer-a*, and *Autographa precationis* showed similar symptoms, particularly spasms and temporary immobility (Dussourd and Hoyle, 2000). Secondly, there was a clear reverse correlation between the performance of monarch larvae on milkweed leaves and the existence of latex and cardenolides (Rasmann et al., 2009). There

are great variations in the concentrations of cardenolides in latex among milkweeds; the weight gain of the larvae of monarch butterfly, *D. plexippus*, a milkweed specialist, was greatest when the larvae were fed the milkweed species that contained the smallest amount of cardenolides (*A. angustifolia*), and the weight gain was smallest when the larvae were fed the milkweed species that

contained the largest amount of cardenolides (*A. barjonifolia*) (Rasmann et al., 2009). Further, monarch larvae grew faster on the cut leaves of *A. angustifolia* that did not exude latex than on intact leaves that exuded latex, and grew slower on the cut leaves painted with digitoxin, a hydrophobic cardenolide (Rasmann et al., 2009). Third, in some milkweed species, such as *Asclepias fascicularis*, an inducible defense trait such as an increase in the amount of latex exudation was observed when the plant individuals were attacked by monarch larvae or were treated with jasmonic acid (JA) (Rasmann et al., 2009). Fourth, a very efficient convergent molecular adaptation was recently found in several milkweed specialist insects from distant lineages (Fig. 4B) (Holzinger et al., 1992; Holzinger and Wink, 1996; Labeyrie and Dobler, 2004). As described earlier, cardenolides exert their toxicity by inhibiting Na^+/K^+ -ATPase, a membrane protein existing in animal cells. It has been known that the Na^+/K^+ -ATPase of several milkweed specialists are insensitive to or not inhibited by cardenolides (Vaughan and Jungreis, 1977). However, the detailed mechanisms for this insensitivity have been unknown. Holzinger et al. (1992) found that in the Na^+/K^+ -ATPase of monarch butterflies, *D. plexippus*, asparagines (Asn) in position 122 of the ouabain (cardenolide) binding site of sensitive enzymes, which consists of 12 amino acids, is substituted by histidine (His) (Fig. 4B). Those authors hypothesized that this mutation accounts for the insensitivity of the monarch enzymes. Later they performed a sophisticated experiment to prove that the substitution of a single amino acid, Asn, by His in position 122 in Na^+/K^+ -ATPase accounts for the ouabain insensitivity of this enzyme (Holzinger and Wink, 1996). They transfected human embryonic kidney cells (HEK), which are sensitive to ouabain, with a mutated *Drosophila* Na^+/K^+ -ATPase gene whose Asn 122 was substituted with His 122, or with the wild-type (unmutated) *Drosophila melanogaster* Na^+/K^+ -ATPase gene, which is sensitive to enzyme inhibition by ouabain (Fig. 4B bottom). When treated with ouabain, the HEK cells transfected with the mutant gene tolerated ouabain and proliferated well, whereas the untransfected HEK cells and the HEK cells transfected by an unmutated gene showed high mortality levels and slow proliferation (Holzinger and Wink, 1996). These results clearly indicated that the single amino acid mutation in Na^+/K^+ -ATPase enables insects to survive on cardenolide-containing plants. More surprisingly, the exact same mutation of Asn122 to His122 in Na^+/K^+ -ATPase took place in several leaf beetle species that belong to *Chrysochus* (Coleoptera, Chrysomelidae) that feed on cardenolide-containing plants (Fig. 4B) (Labeyrie and Dobler, 2004). Two species in this genus, *Chrysochus auratus* and *Chrysochus cobaltinus*, which feed on the cardenolide-containing plants *Asclepias* (Apocynaceae) and *Apocynum* (Apocynaceae), had Na^+/K^+ -ATPase with His in position 122; on the other hand, two other species from the same genus, *Chrysochus asclepiadeus* and *Chrysochus chinensis*, which feed on Apocynaceae plants (*Vinceroxicum*, *Cynanchum*, and related genera) lacking cardenolides, had Na^+/K^+ -ATPase with Asn in position 122 (Fig. 4B). These results show an interesting but rare example of the convergent evolution of physiological adaptive strategies in specialists that feed on plants with a chemical defense. Another rare example can typically be seen in the convergent evolution of secretion of the free amino acids in digestive juice in specialist insects from diverse lineages feeding on the privet tree, *Ligustrum obtusifolium* (Oleaceae), which has counter-adaptive roles against iridoid glycoside-based plant defense (Konno et al., 1997, 1999, 2009, 2010). Although the detailed mechanisms are unknown, Na^+/K^+ -ATPase of some other specialist species that feed on cardenolide-containing Apocynaceae also showed insensitivity. The target-site insensitivity, however, is not the only physiological adaptation to cardenolides; the Na^+/K^+ -ATPase of the oleander hawk moth, *Daphnis nerii*, is highly sensitive to cardenolides, although its larvae can tolerate high concentrations of cardenolides

physiologically (Petshenka and Dobler, 2009). The general existence of physiological adaptation in specialist insects feeding on Apocynaceae that exude latex rich in cardenolides would, in itself, be a strong piece of evidence that cardenolides in latex function as a defense against herbivore and impose selective pressure on herbivorous insects, since for the new adaptive trait to evolve, to be selected for, and to prevail in the insect population, there must have been selective pressures favorable to individuals with those new adaptive traits (e.g., Na^+/K^+ -ATPase with His 122) and unfavorable to those with old traits (e.g., Na^+/K^+ -ATPase with Asn 122).

Recent studies suggested that not only cardenolides but also defense proteins exist in the latex of Apocynaceae species (Ramos et al., 2007, 2010). Latex proteins of *C. procera*, which contained cysteine protease, chitin-binding, and chitinase activities, showed toxicities against a wide variety of pest species including *Anticarsia gemmaralis* (Lepidoptera: Noctuidae) and *C. maculatus* (Coleoptera: Bruchidae) (Ramos et al., 2007, 2010). It is interesting that the larvae of the monarch butterfly, *D. plexippus*, an Apocynaceae specialist that utilizes *C. procera* as a host plant, were completely resistant to the toxicity of latex proteins of *C. procera* (Pereira et al., 2010). Monarch butterfly larvae can even digest the latex proteins of *C. procera* and grow faster when latex proteins are added to the diet (Pereira et al., 2010), while digestive enzymes of *C. maculatus* cannot digest most latex proteins that show toxicity (Ramos et al., 2010).

Now it is clear that three factors of Apocynaceae latex—cardenolides, defense proteins, and stickiness produced by rubber—all contribute to the anti-herbivore plant defenses of Apocynaceae plants.

All of the above observations clearly show that plant latex and its ingredients—chemicals and proteins—play crucial roles in the defense of latex-exuding plants, and in the plant–insect interactions between latex-exuding plants and insects attempting to feed on them. In other words, it is evident now from the above cases that, in order to understand the interaction between latex-exuding plants and herbivorous insects, it is indispensable to know about latex and the defense chemicals and defense proteins it contains.

4. Chemicals and proteins found in latex and their confirmed and/or possible defense activities against herbivores

A great variety of chemicals (mostly secondary metabolites but some primary metabolites) and proteins have been found from plant latex and other exudates (Table 1). Some latex ingredients have confirmed defensive functions against herbivores, some do not have any known or suggested functions, and the rest have unconfirmed but possible defense activity against herbivorous insects.

4.1. Chemicals

4.1.1. Alkaloids

Alkaloids are alkaline compounds that contain nitrogen typically within a ring structure. They are often found in the latex of various plants, many of which are toxic to animals, typically affecting neurotransmission. Alkaloids are found in the latex of several families, including Papaveraceae, Campanulaceae, Apocynaceae, and Moraceae. The opium poppy, *P. somniferum* (Papaveraceae), exudes white latex that contains up to 5% (fresh weight) or 25% (dry weight) morphine (Itenov et al., 1999; Hartmann, 1991), and *C. majus* exudes yellow transparent latex that contains isoquinoline alkaloids such as chelidonium, sanguinarine, and copticine, which together make up 20% (fresh weight) of latex (Tomè and Colombo, 1995). Morphine binds to and activates opioid receptors, which are transmembrane-spanning G protein-coupled receptors, in the central nervous system of humans (Waldhoer et al., 2004), and

sanguinarine affects neurotransmission by inhibiting various neuroreceptors, choline acetyl transferase and DNA synthesis, making sanguinarine toxic to both insects and vertebrates (Schmeller et al., 1997). Lobeline, a piperidine alkaloid, was found from the latex and leaves of *Lobelia cardinalis* (Campanulaceae) (Oppel et al., 2009) and acts on nicotinic acetylcholine receptors (Felpin and Lebreton, 2004). Lobeline has been shown to induce the trenching behavior of the cabbage looper, *T. ni* (Dussourd, 2003). Further, it has been shown in *L. cardinalis* that the trenching by the pink-washed looper, *Enigmogramma basigera* (Noctuidae), causes dramatic change in the distribution of lobeline within a leaf; significant amounts of lobeline accumulate around the trench (Fig. 5F) (Oppel et al., 2009). As described above, the latex of mulberry trees, *Morus* spp. (Moraceae), contains sugar-mimic alkaloids (iminosugars) such as D-AB1 and DNJ, up to 2.5% fresh latex (Fig. 3E; Konno et al., 2006), that are toxic to insects because they inhibit several glucosidases (Hirayama et al., 2007). Apart from this, the latex of some *Ficus* species contains phenanthroindolizidine alkaloids (PIAs) in concentrations high enough to kill generalist herbivores (Konno et al., unpublished).

4.1.2. Terpenoids

The latex of cultivated lettuce, *L. sativa* (Asteraceae), contains several sesquiterpene lactones including lactucin as a complex mixture and the total concentration of sesquiterpene lactones reached 147.1 mg/ml latex (Sessa et al., 2000). Among these sesquiterpene lactones, lactucopicrin and 8-deoxylactucin deter feeding by locusts (Rees and Harborne, 1985), and lactucin triggers trenching behavior to *T. ni* (Noctuidae), a caterpillar that cuts trenches facultatively (Dussourd, 2003). The sesquiterpenes in lettuce latex also have antifungal activity. Lettucenin A, which is induced in latex by microorganisms, inhibited the growth of pathogenic *Cladosporium herbarum* (Sessa et al., 2000). This result indicated latex functions as a defense against pathogens as well as against herbivores. The latex of *Euphorbia* species such as *Euphorbia biglandulosa* and related species contains phorbol and its derivatives (Noack et al., 1980) as well as diterpenoids which are toxic to insects and herbivores; they are also tumor-promoting and cause skin inflammation (Gershenson and Croteau, 1991).

4.1.3. Cardenolides

Cardenolides (Fig. 4A) are a group of cardiac-active steroids (and thus are grouped as terpenoids) that inhibit Na^+/K^+ -ATPase and are present in the latex of many Apocynaceae plants including milkweeds (*Asclepias* spp.) and oleander. Cardenolides (toxicarioides) are also found in the latex of a Moraceae species, *Antiaris toxicaria*, in tropical Southeast Asia, and have been used as dart poisons (Carter et al., 1997). The existence of cardenolides in the latex of both Apocynaceae and Moraceae is a very interesting example of convergent evolution. The defensive roles of cardenolides in latex against herbivorous insects have already been described above.

4.1.4. Rubber

Rubber (*cis*-1,4-isoprene polymer) is a terpenoid that is very widely found in the latex of various plant species; the latex of some 300 genera from 8 plant families are known to contain rubber (Bushman et al., 2006; Metcalfe, 1967; Mooibroek and Cornish, 2000).

Both stickiness and white color, the two most frequently observed traits of latex, are often caused by the existence of rubber dispersed in fluid as particles. Rubber sometimes exists at very high concentrations in fresh latex (e.g., *H. brasiliensis* (Euphorbiaceae) 44.3%, *Ficus* spp. (Moraceae) 15–30%, *Alstonia boonei* (Apocynaceae) 15.5%, *Parthenium argentatum* (Asteraceae) 8%)

(Mooibroek and Cornish, 2000). Such a convergent trait (i.e., frequent existence of high concentration of rubber in latex of many unrelated families) implies that rubber has a very common important role in latex. The primary role of rubber in latex that is generally accepted at present is to produce stickiness; sticky latex mires whole insects (Dussourd, 1993, 1995) or their mouth parts (Dussourd, 1993). But rubber in latex may also have roles in sealing leaf wounds, which would prevent infection by pathogens and the further drainage of latex.

4.1.5. Phenolics

Phenolics including tannins, lignins, and diphenols (catechol), are known to function as plant defenses. Phenolics are sometimes found in plant latex in large amounts. For example, the latex of the sweet potato, *Ipomoea batatas* (Convolvulaceae) contains high concentrations of hexadecyl, octadecyl, and eicosyl ester of *p*-coumaric acids, which altogether make up more than 3% of fresh vine latex and 10% of root latex of the variety "Jewel" (Snook et al., 1994). The observation that the varieties with higher concentrations of (*Z*)-isomers of C16, C18, and C20 coumarates were less accepted by weevils suggested that these compounds in latex have defensive roles against herbivorous insects (Snook et al., 1994). *Rhus* spp. (or *Toxicodendron* spp. lacquer plant) exude resin, which has a similar appearance to latex in that it is white. The resin contains urushiol, a catechol with a long carbon chain rich in double bonds that is highly reactive and known to cause strong skin irritation (Dawson, 1954).

4.1.6. Furanocoumarins

Furanocoumarins are present in the oil ducts of Apiaceae plants such as parsley, *Petroselinum crispum*, and the wild parsnip, *Pastinaca sativa* (Camm et al., 1976; Wu and Hahlbrock, 1992; Reinold and Hahlbrock, 1997; Chambers et al., 2007), and several enzymes involved in the synthesis of furanocoumarins are detected in the oil duct epithelial cells (Schmelzer et al., 1989). Furanocoumarins have a unique phototoxicity. Under UV radiation, furanocoumarins crosslink both strands of DNA by binding to pyrimidine bases, thereby inhibiting DNA replication and transcription; thus, furanocoumarins are toxic to generalist insects under UV radiation (Berenbaum, 1991). Insects that feed on Apiaceae plants adapt both behaviorally and physiologically. To avoid phototoxicity, the larvae of several lepidopteran species roll leaves and feed inside the rolled leaves, thereby avoiding UV radiation (Berenbaum, 1978). Specialist insects that feed on furanocoumarin-containing plants (Umbelliferae and Rutaceae), such as swallowtail butterflies, detoxify furanocoumarins by P-450 oxygenases (Ivie et al., 1987; Nitaó et al., 2003). Angular furanocoumarins are more difficult to detoxify than linear ones (Wen et al., 2006), and some specialists can feed only on linear furanocoumarin-containing plants. Further, in Umbelliferae plants, chemicals that inhibit the detoxification of furanocoumarins and enhance the toxicity of furanocoumarin by inhibiting P-450 activity, such as methylenedioxyphenyl compounds including myristicin and safrole coexist with furanocoumarin (Wen et al., 2006). These studies not only suggest the existence of complicated plant–insect interactions and coevolutionary relationships between plants and herbivorous insects, but also suggest the important role of oil ducts, oil and its ingredients in plant–insect interactions. This role is similar to the roles that laticifer, latex and its ingredients play.

4.2. Proteins

4.2.1. Proteases

Various types of proteases (or proteinases) exist in the latex of plants belonging to diverse phylogenetic groups. For example, cysteine proteases are found in the latex of Caricaceae, Moraceae, and

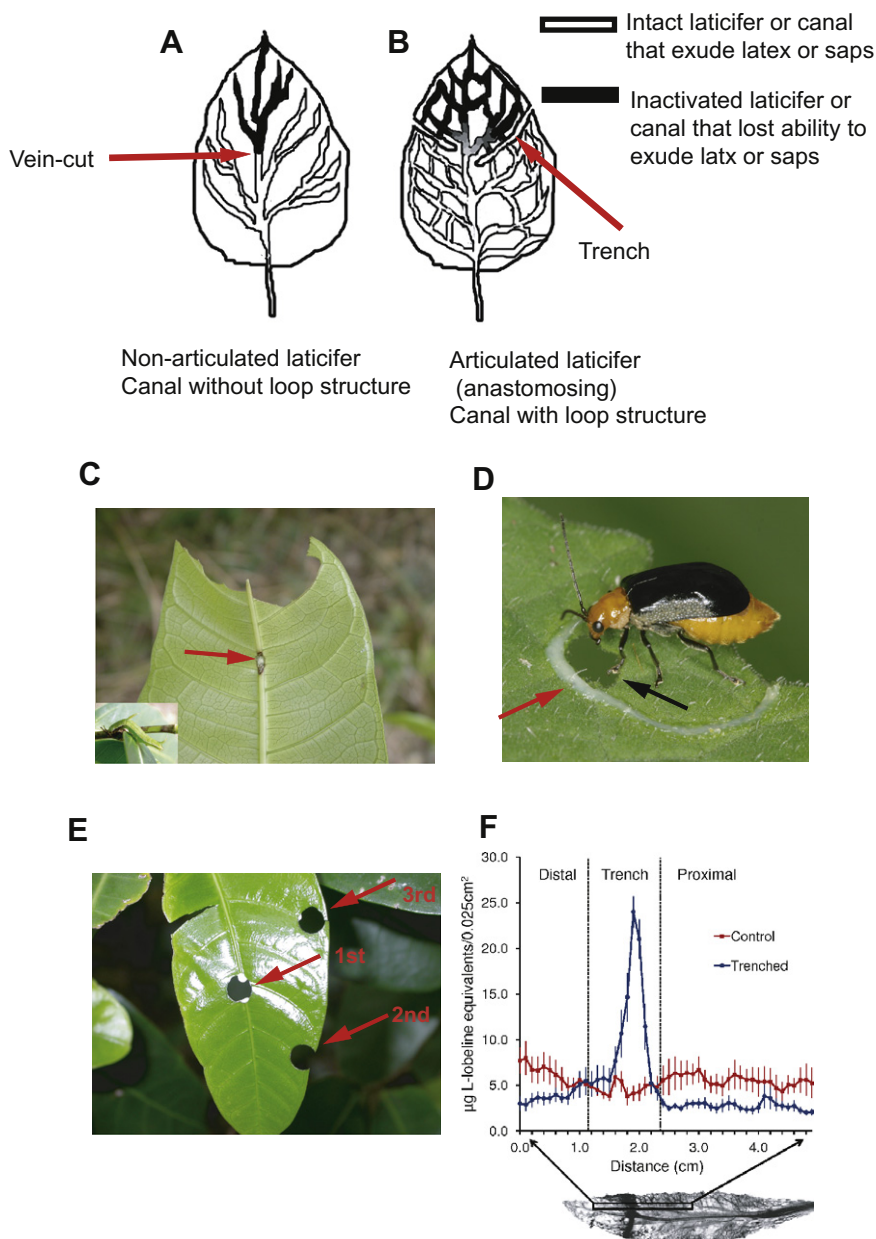


Fig. 5. Vein-cutting and trenching as adaptive behaviors against latex and canal-borne plant defenses, their relationships with the structures of laticifers and canals, and their impacts. (A) Nonarticulated laticifers and exuding canals. These types of laticifers and canals lack bypassing loop structures; a single vein cut can inactivate all of the leaf parts distal to it (downstream). (B) Articulated laticifer (non-anastomosing) and exuding canals with similar structures. These types of laticifers and canals possess numerous bypassing loops and could be inactivated only by cutting trenches that extend across the leaves (distal parts are inactivated) or that surround leaf parts (surrounded parts are inactivated). (C) A vein cut (arrow) made by the larva of a fig specialist butterfly, *Cyrestis thyodamas* (Nymphalidae) (left) feeding on a leaf of the wild fig, *F. virgata*, on Ishigaki Island, Okinawa, Japan. *Ficus* species have nonarticulated laticifers. (D) *Aulacophora nigripennis* beetle, a specialist feeder on Cucurbitaceae plants, feeding within a semicircular trench (red arrow) cut along the leaf edge of a *Trichosanthes cucumeroides* leaf (Cucurbitaceae) in Tsukuba, Japan. A lot of phloem sap was exuded along the trench; no sap is seen where the beetle is feeding (black arrow), suggesting that trenching inactivates exuding phloem in the area surrounded by the semicircular trench (photo provided by courtesy of Prof. David E. Dussourd). (E) Impact of vein cutting on *F. virgata*. To assess the effect of vein cuts on the inactivation of laticifers, three holes were sequentially punched into a leaf attached to a wild-growing *F. virgata* tree on Ishigaki Island, Japan, first on the midvein (arrow 1), then on a lateral vein distal to the first hole (arrow 2), and finally on a lateral vein proximal to the first hole (arrow 3). Ample latex was exuded from the first hole, especially from the proximal rim of the hole, but no latex was exuded from the second hole, indicating that the laticifer in the area distal to the first hole is inactivated by just a single point of damage upstream. Interestingly, latex was still exuded from the third hole proximal to the first hole, indicating that the laticifer in the area proximal to the first damage was not inactivated. (F) Change in the distribution of latex alkaloid in leaves of *Lobelia cardinalis* (Campanulaceae) caused by trenching by a plusiine caterpillar, *Enigmogramma basigera* (Lepidoptera: Noctuidae). The distributions of alkaloids throughout leaves before and after trenching were analyzed by transferring leaf alkaloids to TLC plates, visualizing it by Dragendorff's reagent, and quantifying the alkaloid concentration and distribution by an image analyzer (Oppel et al., 2009). Before trenching, leaf alkaloid was distributed evenly throughout the proximal–distal direction (red line). After the trenching by a plusiine caterpillar, an approximately 50% reduction in alkaloid concentration was observed distal and proximal to the trenching, while a 5-fold increase in alkaloid concentration was observed at the trench. The results visualized the effect of trenching as well as the mobile nature of latex in the laticifer.

Apocynaceae (Kimmel and Smith, 1954; Arribère et al., 1998; Sgarbieri et al., 1964; Ramos et al., 2010; Rasmann et al., 2009), and serine proteases from Moraceae, Euphorbiaceae, Apocynaceae, and

Convolvulaceae (Lynn and Clevette-Radford, 1986a,b; Arima et al., 2000; Tomar et al., 2008; Patel et al., 2007). Both cysteine and serine proteases exist in the latex-like resin of mango,

Mangifera indica (Anacardiaceae) (Saby et al., 2003). The defensive roles of cysteine proteases in latex against herbivorous insects are shown by direct experiments in the papaya tree, *C. papaya* (Caricaceae) and in the fig tree, *F. carica* (Moraceae) as described above (Fig. 1) (Konno et al., 2004). Although the defensive roles of proteases in the latex of other plant systems are not well tested, it is likely that these latex proteases also function as a defense in those plants. The toxic mechanisms of proteases against insects are not well examined except for Mir1-CP, a defense protein with both cysteine protease and chitin-binding activities, which is toxic to insects and accumulates at the site of larval feeding in a maize line resistant to fall armyworm, *Spodoptera frugiperda* (Noctuidae) and other lepidopteran larvae (Pechan et al., 2000). Mir1-CP-fed larvae show degradation of their peritrophic membrane in the insect midgut (Pechan et al., 2002), suggesting that peritrophic membrane is one of the targets of cysteine protease. The dead bodies of young instar *S. ricini* fed papaya and fig latex turned black and soft within 6–12 h, making it almost impossible to pick up the larvae with forceps without breaking their bodies, which suggested that potentially all tissues of insects containing protein could be potential targets of latex proteases (Konno et al., unpublished data). Insects attempting to eat plant leaves were instead digested by the plant!

4.2.2. Protease inhibitors

Protease inhibitors of various types were often detected in the latex of diverse plant lineages. Serine protease inhibitors (trypsin inhibitors) are found in the latex of *F. carica* (Moraceae) (Kim et al., 2003) and *C. papaya* (Caricaceae) (Azarkan et al., 2004). Its gene is expressed in the laticifer of *H. brasiliensis* (Euphorbiaceae) (Han et al., 2000), and cysteine protease inhibitor is detected from the latex of *C. procera* (Apocynaceae) (Ramos et al., 2010). Various types of protease inhibitors including serine, cysteine, and aspartic protease inhibitors exist together in phloem exudates of the pumpkin, *Cucurbita maxima* (Cucurbitaceae) (Kehr, 2006; Walz et al., 2004). In *C. papaya*, a trypsin inhibitor that is absent in undamaged leaves is strongly induced after damage (Azarkan et al., 2004). The defensive roles of protease inhibitors caused by the inhibition of digestive processes are well established in many systems, suggesting that the roles of protease inhibitors found in latex also are defensive. At present, however, there is little direct evidence from bioassays that indicates the defensive roles of protease inhibitors against insect herbivores. One observation that does suggest this would be that the protein fraction of the latex of *C. procera* (Apocynaceae), which has strong cysteine protease inhibitor activity, cysteine-protease activity, chitin-binding activity, and chitinase activity, together showed toxicities to several insects including *A. gemmatalis* (Lepidoptera: Noctuidae), *Ceratitis capitata* (Diptera: Tephritidae) (Ramos et al., 2007), and *C. maculatus* (Coleoptera: Bruchidae) (Ramos et al., 2010). Although the purification of toxic protein has not been completed, the toxicity to *C. maculatus*, whose major digestive protease is cysteine protease, always moves together with cysteine protease inhibitory activity and not with cysteine-protease activity or chitin-binding activity (Ramos et al., 2010). It is likely that the protease inhibitor in latex functions as a defense against herbivorous insects at least in some cases.

4.2.3. Oxidases

Polyphenol oxidase (PPO) and peroxidase (POD) are common plant oxidases, and their existence in latex and resin is reported from Euphorbiaceae, Moraceae, Anacardiaceae, Convolvulaceae, Asteraceae, etc. (Wititsuwannakul et al., 2002; Kim et al., 2003; Saby et al., 2003; Patel et al., 2008; Wahler et al., 2009; Sethi et al., 2009). The fact that latex of many plant species shows browning after exudation and exposure to air suggests that PPO and POD may be widely distributed in plant latex. PPO and POD

are regarded as defense proteins because they convert mono- and di-hydroxyphenol into reactive molecular species as o-quinone, which then covalently bind to nucleophiles such as SH or NH₂ of cysteine and lysine, respectively and make these amino acids unavailable as nutrients (Felton et al., 1992; Zhu-Salzman et al., 2008). Although, conclusive experimental evidence for the involvement of PPO and POD in plant defense against insects is scarce, some suggestive observations exist. The cultivar of romaine lettuce resistant to the banded cucumber beetle (Coleoptera: Chrysomelidae) showed higher constitutive levels of PPO and PAL (phenylalanine ammonia lyase) activity and showed strong induction and much higher induced level of PPO, POD and PAL than a susceptible variety of romaine lettuce that showed no induction (Sethi et al., 2009). In addition to its direct toxicity against insects, PPO is involved in the coagulation of the latex after exudation. When the PPO gene was silenced by constitutive RNA interference in the Russian dandelion, *Taraxacum kok-saghyz* (Asteraceae), the silenced lines exuded four to five times more latex from wounds than the wild-type individuals, and the coagulation rate correlated with the residual PPO activity (Wahler et al., 2009). Proteomic analyses of phloem sap exuded from cucumber, *Cucurbita sativa* and pumpkin, *C. maxima*, revealed an abundance of lipoxygenase (LOX) (Walz et al., 2004). Since LOX is often induced by wounding and/or jasmonic acid, and since reactive hydroperoxides formed by the oxidation of linoleic and linolenic acids by LOX are reported to react with and destroy amino acids essential for insects in addition to the loss of the essential fatty acids (Felton et al., 1994; Zhu-Salzman et al., 2008), LOX is suggested to have defensive roles against herbivores, although no bioassays have been done to examine the involvement of LOX found in phloem sap of Cucurbitaceae in plant defense against insects.

4.2.4. Lectins, hevein-like chitin-binding proteins, and chitinases

Lectins are a series of carbohydrate-binding proteins that have an affinity for specific sugar moieties, and that often have toxic effects against animals including insects (Van Damme et al., 1998). Several types of lectins that differ in binding specificity have also been found in latex from Euphorbiaceae, Moraceae, Apocynaceae, and phloem sap of Cucurbitaceae (Lynn and Clevette-Radford, 1986c; Gidrol et al., 1994; Broekaert et al., 1990; Read and Northcote, 1983; Ramos et al., 2007; Wasano et al., 2009). Hevein, the major latex protein from the rubber tree, *H. brasiliensis* (Euphorbiaceae), is a chitin-binding protein involved in coagglutination of rubber particles (Gidrol et al., 1994) and therefore may be involved in producing the stickiness that mire insects. Its mRNAs are induced by wounding (Broekaert et al., 1990). It is suggested that when *H. brasiliensis* exude latex, rubber particles in the latex covered with receptor proteins glycosylated with polysaccharides rich in GlcNAc (chitin-like polysaccharides) are connected by hevein proteins that have binding specificities to GlcNAc and chitin-like polysaccharides (Gidrol et al., 1994). PPII, another lectin that also has binding specificity to GlcNAc but does not have structural similarity to hevein (Van Damme et al., 1998), exists as a major protein in phloem exudates of Cucurbitaceae plants (Walz et al., 2004; Kehr, 2006) and is involved in the coagulation of phloem sap (Read and Northcote, 1983); PPII binds with another major phloem protein, PPI, and forms insoluble filaments, which cause the coagulation of sap (Read and Northcote, 1983). Coagulation of cucurbit sap has been demonstrated to glue beetle mouthparts (McCloud et al., 1995).

Recently, another chitin-binding chimeric protein, MLX56, which is toxic to insect and which has an extensin domain existing between two hevein domains in the N-terminal region and inactive chitinase in the C-terminal region was found in the latex of mulberry latex as described above (Fig. 3F) (Wasano et al., 2009). Chitin-binding proteins (lectins) with hevein-like domains, such as

wheat germ lectin (or wheat germ agglutinin WGA, which consists of eight hevein domains), are toxic and inhibit the peritrophic membrane in the insect midgut (Hopkins and Harper, 2001).

Chitinases that degrade chitin are widely found in latex from several plant families, including Caricaceae, Moraceae, Apocynaceae, and Euphorbiaceae (Howard and Glazer, 1969; Glazer et al., 1969; Lynn and Clevette-Radford, 1987a; Ramos et al., 2007; Kitajima et al., 2010). The expression of chitinase in the latex of *F. carica* (Moraceae) and *C. papaya* (Caricaceae) increases in response to wounding or treatment with jasmonic acid (JA, the plant hormone involved in the induction of defense responses against herbivory) (Azarkan et al., 2004; Kim et al., 2003). Because chitin is a major component of various insect tissues such as cuticles of the body surface, cuticles of the foregut and hindgut, and the peritrophic membrane in the midgut, and because chitinases from insect origins show toxic effects on other insects when orally ingested (Kramer and Muthukrishnan, 1997; Kabir et al., 2006), it is possible that chitinases in latex would have defensive roles against herbivorous insects. Nonetheless, the toxic effects and defensive roles of plant chitinases are not well established, with a few exceptions. One exception is the chitinase of poplar tree, WIN6, which is induced in poplar leaves in response to herbivory (Lawrence et al., 2006), and which inhibited the growth of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera), when expressed in tomato leaves (Lawrence and Novak, 2006). Another exception is a protein found in mulberry latex that is reported to have chitinase activity and that shows toxicity to the larvae of *D. melanogaster* (Diptera) (Kitajima et al., 2010). Still, it unclear whether or not the defensive activities of these two chitinases come from the “chitinase activities” of these “chitinases”. This is because both of these chitinases, WIN6 and mulberry latex chitinase, are suggested to have both catalytic domains for chitinase activities and hevein-like chitin binding domains (Davis et al., 1991; Kitajima et al., 2010; Van Damme et al., 1998), and because the hevein domain alone shows toxicity, which is evident from the toxicity of WGA consisting only of eight hevein domains. Whether chitinase domains and/or the hevein domains of these chitinases are responsible should be clarified in the future. Because chitin is a major constituent of cell walls in fungi, chitinases in plant latex may also have defensive roles against fungal pathogens.

4.2.5. Others

In addition to the proteins described above that are reported in many plant families, there are still more latex proteins with a limited distribution among plant taxa that may potentially be involved in plant defenses against herbivores. These include lipases in the latex of Caricaceae, Euphorbiaceae, Apocynaceae (Fiorillo et al., 2007; Gandhi and Mukherjee, 2000; Giordani et al., 1991); glutamyl cyclase in Caricaceae (papaya) (Azarkan et al., 2004; Zerhouni et al., 1998); gum Arabic glycoprotein found from exudates of *Acacia senegal* (Fabaceae) (Goodrum et al., 2000); phenylalanine ammonia lyase (PAL) in the latex of romaine lettuce, *L. sativa* (Asteraceae), which is induced after insect herbivory in an insect-resistant line (Sethi et al., 2009); and phosphatase in the latex of Euphorbiaceae (Lynn and Clevette-Radford, 1987b). Phosphatase activities of VSPs (vegetative storage proteins), which are widely distributed in plants such as soybean and *Arabidopsis thaliana*, are associated with the toxic effects of VSPs against coleopteran and dipteran insects, suggesting that phosphatases could potentially function as defense proteins (Liu et al., 2005). Linamarase, a β -glucosidase that specifically degrades linamarin, exists in the latex of cassava, *Manihot esculenta* (Euphorbiaceae). Linamarase is highly concentrated in latex and linamarase activity was more than 300-fold higher than that in its leaves (Nambisan, 1999).

Since insect herbivores that try to eat cassava must consume linamarase in latex and linamarin together, and since linamarase degrades linamarin and forms cyanide, which is highly toxic to a wide variety of organisms including insect herbivores, it is very likely that the latex of cassava and the linamarase in it plays important defensive roles against herbivorous insects.

4.2.6. Indigestibility (hyperstability) or digestibility of defense proteins by digestive enzymes of insects

For plant proteins ingested by herbivorous insects to function as defense proteins, these proteins should tolerate the proteolytic activities of digestive enzymes in the lumens of insect digestive tracts. For example, tomato threonine deaminase toxic to larvae of *Manduca sexta* is stable in the gut lumen and excreted in frass in intact form in *M. sexta*, while other abundant plant proteins, such as Rubisco, that do not have a defensive role are digested and are not detected in frass (Chen et al., 2007). The hyperstability or robustness of defense proteins against digestion by digestive enzymes, which is suggested to be the hallmark of defense proteins (Chen et al., 2007), is also observed in latex proteins which are suggested to have defensive roles against insect herbivory. For example, a chitin-binding defense protein, MLX56, in mulberry latex (see above for detail) shows extreme hyperstability and remains completely intact after incubation with the digestive juice (midgut fluid) of Lepidoptera larvae, bovine trypsin, or bovine chymotrypsin for 24 h at 37 °C (Wasano et al., 2009). Another interesting example of hyperstability comes from defense proteins of *C. procera* (Apocynaceae). A protein fraction of *C. procera* latex, which consisted of several latex proteins, showed strong toxicity (growth inhibition and mortality) to the larvae of *C. maculatus* (Coleoptera: Bruchidae) and *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae). Most latex proteins were not digested by digestive enzymes in gut extracts of both species after 8 h incubation and remained intact. These results suggested that defense proteins of *C. procera* are hyperstable against digestive enzymes of these species (Ramos et al., 2007, 2010). What is interesting is that the latex proteins of *C. procera* did not show toxicity at all to the larvae of the Monarch butterfly, *D. plexippus*, a specialist that can feed on *C. procera*. Rather, the gut protease extracted from the gut of *D. plexippus* larvae digested the “hyperstable latex proteins” completely and immediately, and the latex protein even enhanced the growth of *D. plexippus*! (Pereira et al., 2010). These results indicate that the indigestibility (hyperstability) and/or the digestibility of defense proteins in plant latex by digestive enzymes of herbivorous insects may be a very important factor that determines plant–insect interaction mediated by plant latex.

5. Adaptations of specialist insects to latex-borne defense

5.1. Behavioral adaptations

Many insect herbivores that specialize in feeding on plants that exude latex, oil, resin, and phloem sap, all of which have defensive activities against herbivory, often show a series of adaptive traits based on the same principle. As a pressurized transport system (see Section 8), laticifer and other canal systems are expected to be vulnerable when routes in the proximal areas (upstream) are destroyed. In such cases, the leaf area distal to (down stream from) the point of destruction has no supply of latex from the main reservoir of latex in the proximal parts and also loses pressure to exude latex. In practice, many herbivorous insects, including Lepidoptera, Coleoptera, and Orthoptera, specialize in feeding on plants that have exuding canals, such as laticifer, oil ducts, resin ducts, and phloem of Cucurbitaceae plants have developed vein-cutting and/or trenching behavior (Fig. 5) (Dussourd and Denno, 1991;

Chambers et al., 2007; Dussourd, 2009). The structure of nonarticulated laticifers without bypassing loops (Fig. 5A) suggest that they are more vulnerable to attack, since one disruption upstream in a vein, where laticifers are abundant, can inactivate all downstream laticifers (Fig. 5A, C and E), and it is also suggested that trenching, which cuts all the laticifer throughout the leaves, is necessary to inactivate articulated laticifers that have bypassing loops (Fig. 5B). As suggested, there is a very clear tendency for herbivores that feed on nonarticulated laticifers to show vein-cutting behavior and a tendency for those that feed on articulated laticifer to show trenching behavior (Fig. 5C and D) (Dussourd and Denno, 1991). It has been shown in experiments that artificial and/or insect-made vein cuts and trenching decreased the exudation of latex from the area distal to the cut and trenches (Dussourd and Denno, 1991; Dussourd, 1999, also see photo in Fig. 5E), changed the distribution of latex and lowered the concentration of latex or oil distal to the trench (Fig. 5D and F) (Oppel et al., 2009; Chambers et al., 2007), and dramatically improved the performance of generalist herbivorous insects and slugs that have not adapted to latex (Dussourd and Eisner, 1987; Dussourd and Denno, 1994; Kniep, 1905), whereas without vein cutting and trenching, the insects and slugs eventually cannot grow at all. These results not only show the dramatic adaptive effects of vein-cutting and trenching, but also show the effectiveness of latex-borne and canalicular defenses against herbivores without vein-cutting and trenching behavior. It has been reported that the trenching behaviors of the cabbage looper, *T. ni* (Lepidoptera: Noctuidae), a generalist feeder that often feeds on laticiferous plants and opportunistically shows trenching behavior, is triggered by the existence of latex or phloem saps exuded by several plant species belonging to Apiaceae, Asteraceae, Cucurbitaceae (Dussourd and Denno, 1994), and Campanulaceae (Dussourd, 2003). Further, lactucin from the latex of lettuce, *L. sativa* (Asteraceae), myristicin from the oil of parsley, *P. crispum* (Apiaceae), and lobeline from the cardinal flower, *L. cardinalis* (Campanulaceae), are found to trigger the trenching behavior of *T. ni*. However, Cucurbitacin E from the sap of Cucurbitaceae plants and furanocoumarin from parsley oil, both of which show noxious effects on the performance of herbivorous insects, did not triggers trenching by *T. ni* (Dussourd, 2003). Together, these observations suggest that laticifer destruction (i.e., vein-cutting and trenching) is a general and reliable adaptation to counter latex-borne and other canalicular defenses, which also suggest the general weak point of the latex based on the fundamentals of laticifer as a transport system. Not all specialists adapt to laticiferous plants by disrupting the laticifers; some avoid it instead. For example, several tortoise beetles, *Deloyala guttata*, *Charidotella bicolor*, and *C. purpurata*, which feed on Convolvulaceae plants, feed between the major leaf veins in which the canals are concentrated (Dussourd and Denno, 1991).

5.2. Physiological adaptations

While behavioral adaptations, vein-cutting, and trenching are very common, some insects developed physiological adaptations to defense substances in latex. As described above (Section 3.2.1), several specialist insects feeding on Apocynaceae plants, such as larvae of the monarch butterfly, adapted to cardenolides by developing a Na⁺/K⁺-ATPase insensitive to cardenolides by a single amino acid mutation (Fig. 4B) (Holzinger et al., 1992; Holzinger and Wink, 1996; Labeyrie and Dobler, 2004), although monarch larvae also employ vein-cutting behavior. The monarch larvae seem to have also adapted to the defense protein in latex of Apocynaceae by developing the ability to digest defense proteins (Pereira et al., 2010). The silkworm, *B. mori*, a mulberry specialist, developed sucrase and trehalase insensitive to sugar-mimic alkaloids that are abundant in mulberry latex (Fig. 3) (Hirayama et al.,

2007; Daimon et al., 2008) (see Section 3.1.2). Specialist insects such as the black swallowtail, *Papilio polyxenes*, and the parsnip webworm, *Depressaria pastinacella*, which feeds on Apiaceae that exude oils from oil ducts rich in furanocoumarins, developed the ability to detoxify furanocoumarins with cytochrome P-450 monooxygenase (Ivie et al., 1987; Nitao et al., 2003; Wen et al., 2006). The cabbage looper, *T. ni* (Li et al., 2009), which often feeds on latex-exuding plants, can tolerate cysteine-protease activity, which is often found in latex; cysteine protease inhibitor activity exists in the digestive juice that inhibits cysteine-protease activity and prevents proteins of the peritrophic membrane from being digested (Li et al., 2009). Apart from the above cases, the physical adaptations to defense substances are not well studied, but in the future physiological adaptations will be found for diverse defense substances in latex.

6. Induction and synthesis

6.1. Qualitative induction: induction of defense-related chemicals and proteins in plant latex in response to plant damage and herbivory

A lot of observations suggest that the induction of defense-related substances, especially defense proteins is a common phenomenon (Zhu-Salzman et al., 2008). Latex proteins, many of which are suggested to have defensive roles against herbivorous insects and pathogens, also show induction in response to mechanical damage and insect herbivory. For example, in the papaya tree, *C. papaya*, three enzymes – a trypsin inhibitor, a class-II-chitinase, and a glutamyl cyclase, which are absent in the latex exuded from fully grown immature papaya fruits injured for the first time – are strongly induced and exist in the latex exuded from immature fruits regularly tapped (Azarkan et al., 2004). Among these enzymes, trypsin inhibitor and chitinase are reported to play defensive roles in other plant species, and are reported to be induced by mechanical damages, herbivory, and/or treatment with phytohormones (Zhu-Salzman et al., 2008), although the role of glutamyl cyclase has not been elucidated. Similarly, in the fig tree, *F. carica*, a 10-fold increase in gene expression was observed in three genes for latex proteins – peroxidase, trypsin inhibitor, and chitinase – when the fig trees were wounded (Kim et al., 2003). While the induction of chitinase and peroxidase gene expression was triggered by salicylate (SA), a phytohormone that is involved in anti-pathogen plant defenses, the induction of trypsin inhibitor gene was induced by jasmonate (JA), a phytohormone involved in anti-herbivore plant defenses (Kim et al., 2003). In romaine lettuce, *L. sativa* (Asteraceae), the cultivar highly resistant to the banded cucumber beetle, *D. balteata* (Coleoptera: Chrysomelidae), the activities of three enzymes—phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POX)—significantly increased (1.5- to 2-fold) after plant damage by the beetles (Sethi et al., 2009). The latex exuded from damaged plants showed increased deterrence and browning. These three induced enzymes may be involved in the defense of lettuce through the destruction of nutrients (Felton and Gatehouse, 1996, PPO and POX), the synthesis of defense chemicals (Sethi et al., 2009; PAL involved in phenylpropanoid synthesis), and/or the coagulation of latex (Wahler et al., 2009; PPO see Section 4.2.3). The mRNA expression of hevein, a major protein in the latex of the rubber tree, *H. brasiliensis*, with chitin-binding activity, was reported to increase significantly after wounding (Broekaert et al., 1990). Defense substances that are active against pathogens can also be induced. For example, lettucenin A, a terpenoid that shows strong growth inhibition against *C. herbarum*, was induced in the latex of lettuce, *L. sativa*, when the lettuce plant was treated with CuSO₄ (Sessa et al., 2000). These results, which show the induction of major defense substances

against insect herbivores and pathogens by treatments including mechanical damage and damage by herbivores, strongly support the idea that defensive roles against herbivorous insects and pathogens are major functions of latex.

6.2. Quantitative induction: variation and plasticity in the amount of latex exuded from the wound

Plants not only react qualitatively to insect herbivory by inducing the components of latex as described above; in some cases the amount of latex exudation increases in response to insect herbivory. For example, herbivory by larvae of the monarch butterfly (*D. plexippus*), a milkweed specialist, resulted in approximately a 2-fold increase in latex exudation in milkweed species such as *A. fascicularis* (Rasmann et al., 2009; Agrawal and Konno, 2009) and *A. syriaca* (Bingham and Agrawal, 2010). Herbivory by another specialist caterpillar *Euchaetes egle* (Arctiidae), also increased the amount of latex exudation in *A. syriaca*, but the induced amount was much smaller (1/4) than when the plant was damaged by the monarch caterpillar, *D. plexippus* (Bingham and Agrawal, 2010). In the same plant individual, the amounts of exuded latex are large in younger leaves and tissues near the apex. In sweet potato, *I. batatas* (Convolvulaceae), four times more latex was exuded from cut vines near the apex (young tissue) than from vine cuts near the bottom (old tissue) (Data et al., 1996). This rule seems to hold in many other latex-exuding plants. For example much more latex is exuded from the young shoots, leaves, and petioles of mulberry trees, *Morus alba*, *M. bombycis*, and *M. australis*, and those of figs trees such as *F. carica*, *F. virgata*, and *F. erecta*, than from the old shoots, leaves, and petioles of these plants (Konno, unpublished observation). It seems likely that ample latex in young leaves (latex-borne defense in young tissues) and toughness and high concentrations of phenolics such as tannins and lignins (quantitative defenses and physical defenses in old tissues) complement each other. In other words, such complementary relationships between latex and other defensive traits against herbivorous insects would be reasonably explained only if we suppose defensive roles in latex against herbivorous insects.

6.3. Synthesis of latex ingredients

Synthetic processes of latex ingredients are well studied in the opium poppy, *P. somniferum* (Papaveraceae), which exudes latex containing high concentrations (up to 5% fresh mass) of morphine. The synthesis of morphine includes several steps that take place in different tissues. The synthetic enzymes involved in the early stages of morphine synthesis are localized in parenchymal cells surrounding laticifer cells; enzymes involved in the late stages are localized in the laticifer, suggesting that the early stages take place in parenchymal cells and that the late stage takes place in the laticifer after the intermediate is transported to it (Samanani et al., 2006; Weid et al., 2004). The mRNAs for a number of defense proteins, such as hevein in the latex of the rubber tree, *H. brasiliensis* (Broekaert et al., 1990), MLX56 in the latex of the mulberry tree, *M. alba* (Wasano et al., 2009), asclepain (cysteine protease) in the latex of the milkweed, *Asclepias fruticosa* (Trejo et al., 2009), and peroxidase, protease inhibitor, and chitinase in the latex of *F. carica* (Kim et al., 2003), often exist amply in latex, which suggests that the mRNAs of these proteins are highly expressed in laticifers and that these defense proteins are synthesized in them. Several lines of evidence suggest that papain in the papaya latex and papain-like proteases in latex of other plant such as milkweeds, *Asclepias* spp., are expressed and exist in laticifer as a preproenzyme, which is stable and inactive, and that, immediately after exudation caused by insect herbivory, preproenzymes are digested and modified into active cysteine protease (Silva et al., 1997; Moutim et al.,

1999; Trejo et al., 2009). Such activation systems probably have developed to avoid self-toxicity of cysteine proteases against plants themselves.

7. Evolutionary aspects: diversity and convergence

7.1. Diversity of latex ingredients within families, genera, and species

In spite of its similar appearance, latex ingredients are sometimes diverse even between closely related species in the same families and genera. Moraceae is a large family with around 1000 species of latex-exuding plants, but the latex ingredients are diverse among the species. For example, the major defense substance in the latex of wild fig, *F. virgata* (Moraceae) is cysteine protease; the painting of leaf surfaces of *F. virgata* with E-64, a cysteine protease-specific inhibitor, can deprive the leaves of defensive activity (Konno et al., 2004). Cysteine-protease activity is also detected in the latex of several other *Ficus* species, but in some *Ficus* species, the latex has no cysteine-protease activity, and phenanthroindolizidine alkaloids exist as defense substances (Konno et al., unpublished). Meanwhile, major defense substances in the latex of mulberry trees (*Morus* spp., Moraceae) that are toxic to insects are sugar-mimic alkaloids such as DNJ and D-AB1 (Konno et al., 2006), and a unique chitin-binding protein, MLX56 (Wasano et al., 2009). These are completely different from the defense substances of *Ficus* species. Further, the compositions (molecular species and concentration) of sugar-mimic alkaloids differ significantly among mulberry populations in the same or in sibling species from different locations in East Asia (Konno et al., 2006). It is clear that defense substances in the latex of Moraceae plants are diverse within a family, a genus, and even within a species. A similar diversity of latex ingredients is observed in the family Apocynaceae and the genus *Asclepias* (milkweeds, Apocynaceae) (Seiber et al., 1982; Rasmann et al., 2009), and also in the genus *Euphorbia* (Euphorbiaceae) (Lynn and Clevette-Radford, 1987a). While high concentrations of cardenolides exist in the latex of *A. curassavica* and *C. procerca*, no cardenolides were detected from the latex of *A. speciosa* and *A. californica* (Seiber et al., 1982). The cardenolide compositions of latex also showed diversity among species with cardenolides (Seiber et al., 1982; Rasmann et al., 2009). The diversity of latex ingredients observed even among closely related species would have resulted from different histories and conditions of plant–herbivore interactions that the species have experienced. Further study is necessary, however, to clarify the evolutionary processes that have caused such diversity.

7.2. Common and convergent features

In contrast to the diversity of latex ingredients among closely related species, latex of plant species distant in phylogeny often share similar or the same ingredients in common that seem to have evolved convergently (Table 1). A very evident example is the frequent existence of rubber molecules (*cis*-1,4-isoprenes polymer) in the latex of Moraceae, Apocynaceae, Asteraceae, etc. (Mooibroek and Cornish, 2000, see Section 4.1.4). It is likely that rubber commonly exists in the latex of distant plant groups, since stickiness is one of the basic and common features necessary for the function of latex (see Sections 4.1.4 and 8.5). Since rubber exists in latex-exuding plants of different groups with completely different laticifer structures, including both nonarticulated laticifers (Moraceae, Apocynaceae) and articulated laticifers, the existence of rubber in latex seems to have evolved convergently in many plant groups.

Cysteine-proteases occur in the latex of several different groups, such as Apocynaceae, Moraceae, and Caricaceae (Kimmel and Smith, 1954; Arribère et al., 1998; Sgarbieri et al., 1964) and serine

proteases are found in Moraceae, Euphorbiaceae, Apocynaceae, and Convolvulaceae (Lynn and Clevette-Radford, 1986a,b; Arima et al., 2000; Tomar et al., 2008). Both cysteine and serine proteases exist in the latex-like resin of mango, *M. indica* (Anacardiaceae) (Saby et al., 2003). Since these proteases occur in plants with different canal structures, nonarticulated laticifers (Moraceae, Apocynaceae), articulated laticifers non-anastomosing (Convolvulaceae), articulate laticifer anastomosing (Caricaceae), and resin ducts (Anacardiaceae), it is likely that the existence of proteases in secretory canals has evolved independently and convergently several times in several groups. It is interesting that proteases, which are not common as a defense protein in plants without latex and which have not been regarded as a defense protein until recently, commonly exist in the latex of various plants and function as a strong defense against herbivores at least in the papaya tree, *C. papaya*, and the fig tree, *F. virgata* (Konno et al., 2004). Possible explanations for this are that it is costly and dangerous to keep high titers of proteases in an ordinary manner dispersed throughout the leaf tissues, but not so if they are kept in the latex. If they are concentrated in the latex, the plant needs to produce smaller total amounts of costly proteases without losing the strong defensive effects; at the same time, the proteases, which are potentially harmful to the plant itself, could be separated (compartmentalized) and safely kept.

7.3. Origins of latex and laticifer

The origins of latex and laticifer are an open question. Since laticifers exist sporadically throughout the plant kingdom, it has been suggested that latex and laticifers have evolved many times independently (Farrell et al., 1991). The considerable differences in the structures and ways of development observed among several types of laticifers (Dussourd and Denno, 1991) support the idea that latex and laticifer have evolved independently and convergently. However, there is no evidence at present concerning how and from what latex and laticifer have evolved.

8. Latex and exudates as defense systems: the mechanistic essence, unique characteristics, advantages, and disadvantages of canalicular defense systems

The frequent convergent evolution of latex (and also exudates such as resin, oil, and phloem sap) in a wide variety of plants distant in phylogeny suggest that there are common advantages in latex-borne defenses and other canalicular defenses. Therefore, I would like to discuss here the mechanistic essence and unique characteristics of latex as a defense system against herbivores. I will also discuss its advantages (Fig. 6B), which may have led to the convergent evolution of latex, as well as its disadvantages, which stem from the fundamental nature of this system, by combining previous discussions on latex-borne defenses (Dussourd and Denno, 1991; Dussourd, 1993) and novel views based on recent observations on latex.

8.1. Existence of various defense substances in latex and exudates in highly concentrated manners

As described in Section 4, latex and exudates contain an enormous variety of defensive chemicals, including toxins and anti-nutritive compounds from all major classes of secondary metabolites and various types of defense proteins (Table 1). Moreover, these compounds exist in latex and exudates in highly concentrated manners. The average concentration of a defense substance in latex is often much higher than that in leaves (and measures of leaf chemistry often include residual contents of

laticifers). Typically, 20–2000 times higher concentrations (compared to leaf average) of defense chemicals or proteins are observed in the latex of milkweeds, lettuce, and papaya. For example, in *A. curassavica* (Apocynaceae), 20–50 times higher concentrations of cardenolides exist in latex than in leaves, and in *C. procerca* (Apocynaceae) the difference is 80 times (Seiber et al., 1982; Groeneveld et al., 1990, 1991); lettuce, *L. sativa* (Asteraceae) has an approximately 2000 times higher sesquiterpene lactones concentration in the latex (Sessa et al., 2000); in *C. papaya* (Caricaceae), approximately 500 times higher cysteine-protease activity occurs in latex (Konno et al., 2004). In mulberry, *Morus* spp. approximately 100 times higher concentrations of sugar-mimic alkaloids are found in latex than in whole leaves (Konno et al., 2006). What the herbivorous insect confronts when attempting to feed on a latex-exuding plant would likely be very high concentrations of defense substances observed in the latex rather than the much lower average concentrations contained in the leaves. When a tiny insect bites the leaf, a relatively large droplet of latex with high concentrations of defense substances emerges immediately at the very places of biting.

8.2. Mobile nature of latex and exudates: latex as a system that transports defense substances to the precise point of damage

Latex is mobilized and transported immediately to the site of damage (Fig. 6A and B) (e.g., latex can travel more than 70 cm to

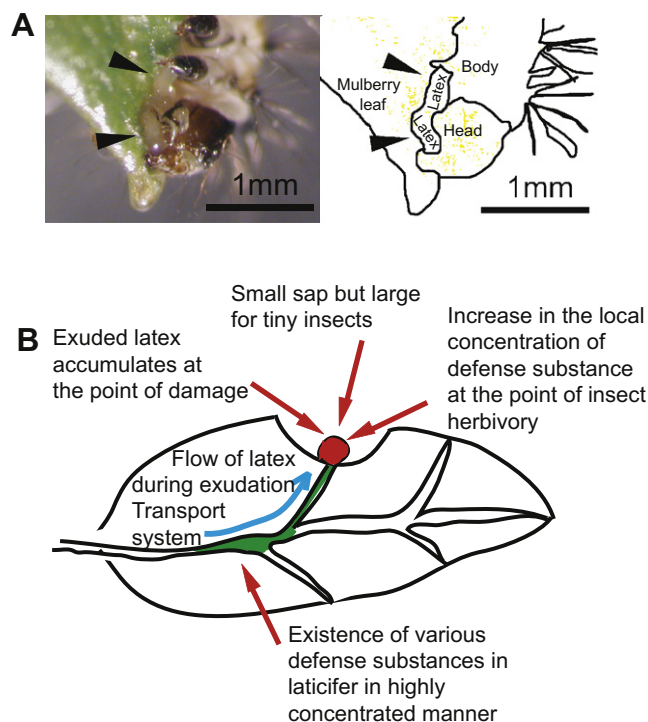


Fig. 6. Mechanistic characteristics of latex-borne and canalicular defenses. (A) A tiny first instar larva of the Eri-silkworm confronting a large amount (compared to its tiny body size) of mulberry latex with high concentrations of defense chemicals (sugar-mimic alkaloids) and protein (MLX56) when eating mulberry leaves (Konno et al., 2006; Wasano et al., 2009). Tiny insect herbivores almost inevitably take up a large amount of concentrated toxins. (B) Schematic model showing the essences of latex-borne and canalicular defenses. The latex with concentrated defense substances exists throughout the laticifer (area shown in green) before insect attack, and concentrates at the point of damage (area shown in red) immediately after the damage. This point is very clear from Fig. 5F, which shows a 5-fold increase in alkaloids after trenching (multiple damages). Since the local concentration of defense substances rises at the point of damage, latex is similar to inducible defense systems, although it is a preformed defense.

the damaged (exuding) points in *Cryptostegia grandiflora* (Buttery and Boatman, 1976). The mobile nature of latex and other canal systems such as oil ducts, after insect herbivory is evident from several recent observations that visualized the change of distribution of defense chemicals after the damage by herbivorous insects (Fig. 5F) (Oppel et al., 2009; Chambers et al., 2007). In *L. cardinalis* (Campanulaceae), which exudes latex that contains lobeline, an alkaloid, the concentrations of lobeline around the trench increased by at least 5-fold after trenching by plusiine caterpillars, *E. basigera* (Lepidoptera: Noctuidae), but the distal and proximal concentrations of lobeline decreased by half before trenching (Fig. 5F) (Oppel et al., 2009). Also, in the wild parsnip, *P. sativa* (Apiaceae), furanocoumarins accumulated around the trenches made by cabbage loopers, *T. ni* (Lepidoptera: Noctuidae) (Chambers et al., 2007). These examples of the mobility of latex come from trenching by latex specialists. But it is reasonable to assume a similar accumulation of exuded latex and a subsequent rise in local concentrations of defense substances in latex around the bites made by generalist feeders that have not developed behavioral adaptations such as trenching and vein cutting (Fig. 6A). Since the local concentrations of defense substances rise at the point of damage (Fig. 6B), latex is similar to an inducible defense system, although it is preformed in the plant. But in the latex-borne defense, the rise takes place immediately after damage – within a few seconds – which is much faster than in an inducible defense in which it takes at least hours or days for the concentrations of defense substances to rise sufficiently. The mobility that enables the immediate transport of defense substances to the precise point of a herbivore attack seems to be a great advantage of latex-borne or canalicular plant defenses.

8.3. Economical system: low defense cost with high efficiency

Points in Sections 8.1 and 8.2 above indicate that latex is an economical system that provides a concentrated defense when and where it is needed most with a relatively small total amount of defense substances deployed. In laticiferous plants, the total costs for the plant to produce defense substances are what is estimated from the average concentrations in whole leaves, while the actual defensive effects against herbivorous insects (the concentrations of defense substances that the herbivorous insects encounter) are estimated from the concentrations in latex, which are typically 20–2000 times higher than those in whole leaves (Seiber et al., 1982; Groeneveld et al., 1990, 1991; Sessa et al., 2000; Konno et al., 2004, 2006). In the ideal case in which insects are forced to drink pure latex, a latex-borne defense is 20–2000 times more economical than an ordinary defense with an even distribution of defense substances throughout the leaves. In reality, herbivorous insects may consume, say 10% latex and 90% leaf, and then the rate goes down to 2–200 times. Nonetheless, latex-borne defense is 2–200 times more advantageous in terms of production costs. This economy would explain why latex is advantageous and successful as a defense against herbivory, and it would explain why latex and other exudates evolved convergently in so many distant phylogenetic groups, and why those groups that evolved latex-borne and other canalicular defenses are more flourishing and contain more species than their sister groups that did not evolve canalicular defenses (Farrell et al., 1991).

8.4. Effectiveness of latex-borne defense to tiny herbivores

Given the points in Sections 8.1 and 8.2 above, latex seems to be most effective against tiny chewing insects. As described in Section 8.2, when tiny insects bite in leaves of laticiferous plant, a sap comparatively large to the size of the tiny insects appear in front of the insects (Fig. 6A) and they are forced to take up rela-

tively large amounts of latex and small amounts of leaves; in this case, the tiny insects confront the high concentrations of defense substances observed in latex. On the contrary, larger herbivores, such as mammals, that feed on whole leaves (or even entire plant) should be much less affected because the accumulation of latex at the site of damage will be ineffective at all. When a large insect herbivore bites a leaf, the amount of sap is relatively small compared with the size of a bite. As a result, a large insect herbivore consumes a relatively small amounts of latex and large amounts of leaf. The concentration of defense substances in that case is closer to the average concentrations in whole leaves. Thus the economic effects described in Section 8.2 no longer apply. This view that the latex-borne defense is more effective against tiny insects than to larger insects is supported by empirical observations. For example, the mortality rates of milkweed specialist caterpillars feeding on milkweeds are high in early instars and extremely high in the first instar, even though the later instar larvae perform well on milkweed (Zalucki et al., 2001a,b).

8.5. Stickiness and clotting function as defense but are also necessary in maintaining the system

Stickiness and/or clotting activity (or coagulation) are interesting features that are very often observed in most latex and exudates and very often coincide. Although both concepts are different from each other, stickiness and clotting activity are closely related and share common features. Clotting is a solidification of fluid and often coincides with increased viscosity, although there are exceptions (freezing, crystallization, and some cases of gelling in which viscosity does not increase). Stickiness is always associated with high viscosity and often with elasticity, but it also must adhere to a surfaces. Stickiness of latex itself functions as a defense against herbivores by trapping the whole body of insects (Dussourd, 1993, 1995) and by gluing the mouthparts of insects (Dussourd, 1993). As discussed above, rubber, which is highly elastic, is involved in stickiness in many laticiferous plants. The mechanisms of clotting and stickiness have been studied in the latex of the rubber tree, *H. brasiliensis* (Euphorbiaceae), and rubber particles and hevein were shown to play important roles in the coagulation of latex (Gidrol et al., 1994), although much remains unsolved, such as the mechanisms of adhesiveness (affinity) to the insect surface. Apart from the direct defensive roles of stickiness, clotting (coagulation) seems to be an indispensable feature of latex and exudates in exerting the defensive effects described in Section 8.2 above. In order for latex to flow inside laticifers and for large amounts of it to exude at the site of damage, it is necessary for the plant to maintain high pressure inside the laticifers. If it were not for the clotting of latex that plugs the damaged points of laticifers, the pressure would decrease rapidly and latex would not flow from wounds made by subsequent bites. After a second damage made soon after the initial damage (trenching), the amounts of latex and oil exuded from laticifers and oil ducts decreased, but the amounts of exudation recovered when further damages were made after long intervals (Oppel et al., 2009; Chambers et al., 2007). These observations suggested that drainage of latex and decreased pressure in laticifers or oil ducts take place after the damage and that plugging should be necessary to restore the pressure. Meanwhile, for a laticifer, which is a thin tube, to function as a transport system, latex must not have high viscosity when it is inside the laticifer. To fulfill these requirements (i.e., to maintain internal pressures, prevent the loss of latex after damage, and maintain mobility within the laticifer) clotting or coagulation seems to be an essential and indispensable feature of latex for its defensive roles against herbivorous insects and against other organisms as well. In reality, clotting occurs within a few minutes after damage and exudation.

8.6. Vulnerability that comes from the fundamentals of latex-borne defense

As described in Section 8.2, the essence of the advantages of latex-borne and other canalicular defense systems come from the fact that latex, laticifers, and exuding canals are the systems that transport defense substances by internal pressure to the point of damage immediately after a herbivore attack. This fundamental feature of laticifers as transport routes and systems, however, makes latex-borne defenses vulnerable to herbivores, since their function is easily lost when laticifers (and oil ducts, resin ducts, and exuding phloem) are disrupted. Indeed, this is the most common counter-defense (i.e., vein-cutting and trenching, see Section 5.1) by latex-adapted herbivores. This strategy is highly efficient and successful and has convergently evolved in many insect groups including Lepidoptera, Coleoptera, and Orthoptera, that feed on plants with canalicular defenses that exude latex, resin, oil, or phloem saps (Dussourd and Denno, 1991; Chambers et al., 2007; Dussourd, 2009).

9. Conclusion and future perspective

It is becoming clear that latex, laticifers, exudates, and exuding canals play very important roles, physiologically, ecologically, and evolutionarily, in anti-herbivore plant defenses and in plant–insect interactions in a very unique way.

It is now apparent that latex contains a great variety of defense chemicals and defense proteins in highly concentrated manners. Most groups of currently known defense substances are included in the repertoire of latex ingredients. Further, a large number of plants species (more than 20,000) exude latex, and latex ingredients are diverse even among closely related species. These facts mean that latex is a treasury of defense substances. The fact that defense substances exist in highly concentrated manners means that defense substances exist in latex half purified, and that it is easy to find targets during purification and identification. It is possible that a lot of novel defense substance will be discovered in latex in future studies.

Latex and laticifers (exuding canals and exudates) are not only the reservoirs of defense substances; they provide very unique mobile mechanisms to transport and deliver defense chemicals to the point of an herbivore attack immediately. This means that while a plant needs to produce only a small total amount (an average concentration) of latex, the concentration that the insect confronts (the concentration in latex, which is the actual defensive effect) would be very high, so the defensive ability of leaves is much stronger than what is suggested from the average concentration in leaves in laticiferous plants. This means that the effects of defense substances that exist in canalicular systems, such as latex and laticifers, can be easily underestimated and/or overlooked if we use the classical extraction and bioassaying methodologies depending on extracting defense substance from whole leaves by solvent or homogenization neglecting the existence of canalicular defense systems. Not all latex and exudates have apparent white color, and not all of them are saps large enough to be visible. Some latex and exudates could be transparent, without color, and very small, and would still be overlooked. But they may still contain high concentrations of defense substances and would play important roles in plant–insect interaction in ways similar to the examples shown in this review. Future studies will unveil much more interesting examples of latex-borne defenses and other similar defense systems, as well as much more interesting defense substances including defense chemicals and proteins.

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Kotaro Konno is a chief researcher at the Division of Insect Sciences in the National Institute of Agrobiological Sciences, Tsukuba, Japan. He received M. Sci. from the University of Tokyo in 1991. He joined the National Institute of Sericultural and Entomological Science, Tsukuba, Japan as a researcher in 1994 and was promoted to the present position in 2001. He received his Ph.D. in agricultural and life sciences from the University of Tokyo in 2002. He is a chemical ecologist who has worked mainly on plant–herbivore interactions mediated by plant secondary metabolites and defense proteins, and on the physiological and molecular adaptation of specialist insect herbivores against plant defenses. He has discovered novel antiherbivore defense mechanisms of privet trees, mulberry trees, papaya trees, and fig trees, and the physiological adaptation mechanisms of specialists feeding on these plants, thereby elucidating an intimate coevolutionary relationship between plants and insects at the molecular level. He is currently interested in studying plant latex as a plant defense system against insect herbivores and has recently identified several defense chemicals and proteins from the latex of several plant species.