

Holey Aroids: Circular Trenching Behavior by a Leaf Beetle in Vietnam

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ABSTRACT

The leaf beetle *Aplosonyx ancora* cuts circular trenches on the underside of leaves of their aroid host plants before feeding. Latex is exuded from these cuts and the beetles feed intermittently on these isolated tissues, apparently avoiding the latex. Circular feeding holes occur only in Araceae in which latex is contained in cells or vessels with cells bridging adjacent files ("anastomosing laticifers"). The phylogeny of Araceae and the restricted host records suggest a coevolutionary arms race and parallel phylogenesis between *Aplosonyx* and its aroid hosts.

Abstract in Vietnamese is available at <http://www.blackwell-synergy.com/loi/btp>.

Key words: Araceae; Chrysomelidae; cyanide; herbivore defense; herbivory; latex.

THE CORRESPONDENCE BETWEEN INSECT FEEDING BEHAVIOR AND HOST PLANT CHARACTERISTICS is one of the clearest examples of how natural selection has shaped insect–plant interactions (reviewed in Dussourd & Denno 1991, Dussourd 1994). Specialized feeding behaviors are usually correlated with host plant defenses and secretory canal architecture. The best studied examples involve temperate plant–insect associations, *e.g.*, herbivores of milkweeds (*Asclepias*) and cucurbits (*Cucurbita*) in response to latex with cardenolides (Dussourd & Eisner 1987, Dussourd 1990, Dussourd & Hoyle 2000, Lewinsohn & Vasconcelos-Neto 2000) and phloem sap with cucurbitacins (Tallamy 1985, McCloud *et al.* 1995), respectively. Here I report a specialized type of feeding by leaf beetles on aroids and interpret these natural history observations in the context of both the vegetative anatomy and phylogeny of the host plants. Circular trenching behavior allows the beetles to avoid the latex of the host plants and to perhaps sequester cyanogenic compounds as defensive allomones. Reciprocal evolution may be occurring between *Aplosonyx* leaf beetles and particular aroid genera, the giant elephant ears (*Alocasia*, *Colocasia*).

The leaf beetles (Chrysomelidae) are almost exclusively phytophagous, and are one of the largest families of Coleoptera with around 40,000 described species (Farrell & Sequeira 2004). There are, however, very few reports of leaf beetles with behavioral counterplays to host defenses (Becerra 1994). Aroids (Araceae) are conspicuous components of the understory herb layer in most tropical forests and leaves can often grow to enormous sizes (up to 4.5 m long and 2.5 m wide in *Alocasia robusta* M. Hotta). In May 2000, at Ba Be National Park (NP) (Cao Bang Province, Vietnam, 22°24' N, 105°37' E, 145 m asl) leaves of *Alocasia navicularis* Koch & Bouche and *C. gigantea* Blume were observed to have nearly perfect circular holes (mean diameter: 32.4 mm, range: 17.1–47.8 mm, $N = 121$; N. Nguyen, pers. comm.; Fig. 1A). Nocturnal observations revealed

that this damage was a result of very precise feeding behavior by *A. ancora* Laboissier (Fig. 1D). Prior to feeding on the leaf tissue of *C. gigantea*, the adult beetles cut a circular trench from the undersurface of the leaves using their mandibles (Fig. 1B). The trench cut minor veins and the leaf epidermis and mesophyll, and encircled an area of leaf up to three times. The cuts initiated the release of latex from the leaf, which subsequently pooled and dripped from its underside (Fig. 1C). The beetles began feeding away from the oozing latex (Fig. 1C) and eventually consumed virtually all of the tissues enclosed by the trench (Fig. 1D). The small pieces of remaining plant tissue eventually withered and fell off the leaf resulting in the near perfect holes in the leaf surface (Fig. 1A).

A review of the literature revealed that these observations are the first host record for *A. ancora*, a species that is restricted to Vietnam and southern China (Gressitt & Kimoto 1963). There are at least 45 species of the galerucine genus *Aplosonyx* distributed from south India to SW China, to the Philippines and Sulawesi (Indonesia) (C. Reid, pers. comm.) and 16 species in Malaysia (Mohamedsaid 2004); all published hosts are Araceae (Reid 1998). Interestingly, field biologists and aroid taxonomists and collectors have observed and photographed holey aroids in southeast Asia but the cause of the damage was not known (C. Reid, P. Boyce, T. Kohei, S. Hyndeman, E. Darling, pers. comm.). Circular trenching has also been documented in *A. albicornis* (Wiedemann) feeding on *Colocasia esculenta* (L.) Schott (photograph by C. C. Lee). To date, circular trenching behavior by beetles has only been reported on Cucurbitaceae: *e.g.*, *Epilachna borealis* Fab. (Coccinellidae) adults (fig. 1 in Tallamy 1985) and larvae (fig. 2c in Dussourd 1999) feeding on *Cucurbita*; *Aulacophora femoralis chinensis* Weise (Chrysomelidae) adults feeding on *Cucumis* (Fig. 1 in Kong *et al.* 2004); and *Aulacophora* spp. adults feeding on a variety of cultivated melon plants (Lewis & Metcalf 1996). There are, however, a number of striking differences in the trenching behavior and subsequent leaf consumption by *Aplosonyx*. The cucurbit feeders make much smaller holes, relative to the size of the beetle, that are more irregular in shape and

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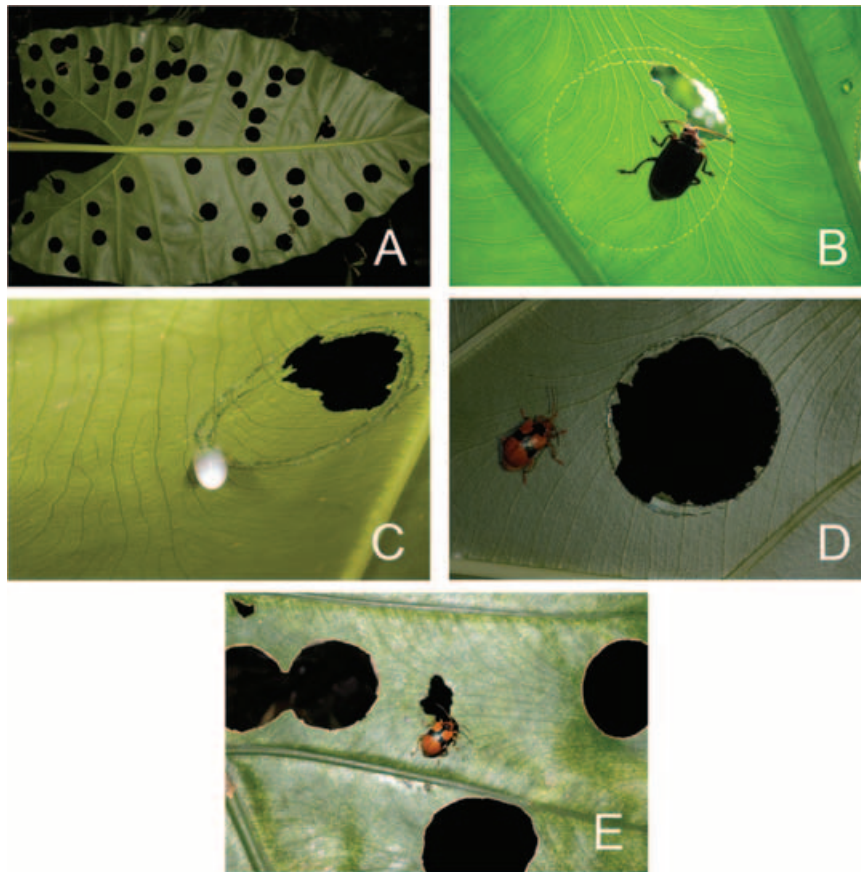


FIGURE 1. Circular trenching of aroid leaves (Araceae) by *Aplosonyx ancora* (Chrysomelidae). (A) Heavily damage leaf of *Alocasia navicularis*. (B) Leaf of *Colocasia gigantea* with circular trenches. (C) Latex exuding from partly eaten trenched leaf of *Colocasia gigantea*. (D) Beetle and almost completely consumed hole on *Colocasia gigantea*. (E) Beetle and feeding damage on heavily attacked leaf of *Alocasia navicularis*. (A, E) Cuc Phuong National Park; (C, D) Ba Be National Park; (B) Bach Ma National Park.

often include the leaf margin. There is never the striking pattern of leaves with many distinct circular holes (Fig. 1A).

Subsequent fieldwork in Vietnam at Bach Ma NP (May 2001, 2003, 2004; Thua Thien-Hue Province, 16°12' N, 107°51' E, 500–1000 m asl), Ba Be NP (June 2004), and Cuc Phuong NP (June 2005; Ninh Binh Province, 05°29'–44' N, 20°14'–24' E, 400–500 m asl) revealed that the association of *A. ancora* and aroids is more complex than initially thought.

At Bach Ma NP, *A. ancora* cuts circular trenches in both *Alocasia navicularis* and an undescribed species of *Colocasia*. There is a temporal variation in the pattern of herbivory. In May 2004 many *Alocasia* leaves had old holes but no beetles were found feeding on this species. The *Colocasia* had recent holes and feeding was observed. These two hosts differ in their physical and chemical attributes. The *Alocasia* had much tougher leaves, and the cut leaves and stems had a stronger smell of cyanide (A. Agrawal, pers. comm.). In one roadside locality erosion exposed the roots of an *Alocasia* and many of the holes were irregular in shape and there was no evidence of trenching (as in Fig. 1E). These irregular holes resemble the feeding damage caused by the beetle when caged with

excised leaves (A. Tran & S. Amato, pers. comm.), suggesting that trenching is only associated with intact leaves on healthy plants, *i.e.*, those with a functioning vascular system.

At Ba Be NP, *Aplosonyx* beetles were commonly observed on *C. gigantea*. Only one *A. navicularis* had a feeding hole, and although circular it was not trenched. A single beetle trenched and consumed at least five holes in a single night (Ba Be NP, 3 June 2004). The beetles are nocturnal but I observed feeding in the early morning hours of an overcast and rainy day.

At Cuc Phuong NP, *A. navicularis* is very common and often grows on limestone boulders. It is heavily trenched by *A. ancora* (Fig. 1A). One beetle fed without cutting a trench; this was a heavily attacked leaf and many of the laticifers (latex containing cells and vessels) had been undoubtedly severed by previous feeding by the beetles (Fig. 1E).

Comparative data from the Araceae suggest that the association between *Aplosonyx* and aroids may represent a new example of herbivores overcoming the defenses of their hosts. This insight derives from two sources—the vegetative morphology of the leaves and their chemical constituents, and the current phylogeny for the

Araceae (Mayo *et al.* 1997). *Alocasia* and *Colocasia* are closely related genera classified in the Tribe Colocasieae (six genera, all Old World tropics). This tribe and the Tribe Caladieae (seven genera, six are restricted to the New World tropics) are considered to comprise the subfamily Colocasioideae (Grayum 1990). Although Araceae are recorded as a latex-producing family (Lewinsohn 1991), laticifers are absent in the basal clades and the Colocasioideae is the only group of aroids characterized by anastomosing laticifers, complex networks of interconnected files of laticifers (*sensu* French 1988). Correlated with the presence of anastomosing laticifers in the Colocasioideae is the only report of sterol ester latex in the monocots (Fox & French 1988).

Dussourd and Denno (1991) documented a precise match between the type of laticifers and insect feeding behavior in a diversity of dicots. Trenching is associated with anastomosing articulated laticifers and latex in the Asteraceae and Caricaceae, and the trenching insects were Lepidoptera larvae (Noctuidae and Sphingidae). The observations reported herein, when coupled with the distribution of the laticifers and latex in Araceae, support the hypothesis that circular trenching behavior in *A. ancora* is another case of deactivation of plant mechanical defenses by a behavioral counterploy.

Farrell and Sequeira (2004) examined evolutionary rates in the Chrysomeloidea and found that changes in host taxon used for feeding (*i.e.*, gymnosperms, monocots, eudicots) were the main cause of ecological diversification. Their phylogenies suggest a Cretaceous origin of external leaf feeding, and shifts to dicot hosts in the Chrysomelinae and Galerucinae. However, the host records for *Aplosomyx* summarized here suggest a further shift to monocots in the chrysomelid subfamily Galerucinae. This shift is to a highly derived clade of aroids with physical and/or chemical defenses, which appears to have required the development of circular trenching behavior.

Farrell *et al.* (1991) used independent phylogenetic contrasts to demonstrate that latex and resin canals are associated with the more species-rich sister group in 13 of 16 lineages of plants. For Araceae they made a high level comparison of subfamilies, contrasting the nonlaticiferous Pothoideae (≤ 995 species) with Aroideae, Philodendroideae, and Colocasioideae (1350 species), which they characterize as lineages with latex. They interpret this as support for the hypothesis, albeit rather weak. However, the current working phylogeny for the Araceae (Stevens 2001) provides much stronger support for their hypothesis. Laticifers are absent in the five basal subfamilies of Araceae and are also absent in the Lasioideae (58 species), which is the sister group to the Calloideae and Aroideae (2666 species) in which laticifers are present and usually anastomosing. The contrast provides strong support for the hypothesis that anastomosing laticifers have increased the rate of speciation in the Araceae.

More complete information on the host associations of herbivores of aroids is required to determine if anastomosing laticifers have had reciprocal effects on other herbivores of Araceae. This report is the only documented case of circular trenching behavior in the Araceae. There are, however, anecdotal natural history observations of New World *Xanthosoma* species with circular holes (Mexico, Los Tuxtlas field station and Palenque, D. Dussourd, pers. comm.;

Panama, Bocas del Toro, D. C. Darling, unpublished). *Xanthosoma* is the most species-rich genus of the Tribe Caladieae (*ca* 57 species, Mayo *et al.* 1997) and has species with both anastomosing laticifers and latex (Fox 1988, Fox & French 1988). The trenching herbivores have not been identified but these observations establish an independent origin of circular trenching because *Aplosomyx* is restricted to the Old World tropics.

Aplosomyx species associated with aroids are brightly colored and have all the hallmarks of aposematic or warning coloration: *A. ancora*, black and red pattern, *A. albicornis*, iridescent blue with contrasting white antennae. The host plants of *Aplosomyx* (*Alocasia*, *Colocasia*) are known to contain the cyanogenic glucoside triglochlinin (Bradbury *et al.* 1995) and hydrogen cyanide is a defensive allomone in some species of leaf beetles (Moore 1967, Pasteels *et al.* 1994). The possibility of aposematic coloration in some species of *Aplosomyx* suggests an exciting new line of inquiry. Differing amounts of cyanide in the leaves (Bradbury *et al.* 1995) may help explain the feeding preferences of *A. ancora* and other aroid herbivores.

There is still a considerable debate in the plant-herbivore literature about the role of trenching and vein cutting (McCloud *et al.* 1995, Dussourd 2005). Are these feeding behaviors responses to chemical and/or mechanical defenses of the host plant (Becerra *et al.* 2001) and do specialist and generalist herbivores respond to these defenses in different ways (Strauss & Zangerl 2000)? The *Epilachma*-cucurbitid association and the diverse community of herbivores on milkweeds have demonstrated the complexities of avoidance, sequestering, and feeding attractants and revealed the dynamic nature of plant-herbivore associations. More complete information on the aroid hosts used by *Aplosomyx* beetles, and chemical analyses of both these herbivores and their host plants, should provide a diverse tropical study system to complement milkweeds and cucurbits.

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