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The lethal plant defense paradox remains: inducible host-plant aristolochic acids and the growth and defense of the pipevine swallowtail

James A. Fordyce
Section of Evolution and Ecology , Center for Population Biology, University of California, Davis, CA 95616, USA (Phone: 530 752-2225; E-mail: jafordyce@ucdavis.edu)

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Abstract

Toxic plants with sequestering specialists are presented with a problem because plant derived toxins protect herbivores against natural enemies. It has been suggested that early induction of toxins and later relaxation of these defenses may help the plant resolve this problem because neonate caterpillars incur the physiological cost of dealing with toxins in early life, but are denied toxins when they are able to sequester them efficiently. In California, the pipevine swallowtail, *Battus philenor* L. (Lepidoptera: Papilionidae), feed exclusively on *Aristolochia californica* Torrey (Aristolochiaceae), an endemic vine that contains toxic alkaloids called aristolochic acids that caterpillars sequester to provide chemical defense in immature and adult stages. In a field experiment, the concentration of aristolochic acids doubled in the plant following leaf damage and returned to constitutive levels after six days. Neonate pipevine swallowtail caterpillars showed no aversion to high levels of aristolochic acid in a preference test. Caterpillars reared on leaves with supplemented aristolochic acid showed no physiological cost or increased mortality compared to caterpillars reared on un-supplemented leaves. Searching efficiency and capture rate of lacewing larvae (*Chrysoperla*), a common predator of first instar caterpillars, was compromised significantly after feeding on caterpillars reared on leaves with supplemented concentrations of aristolochic acid compared to caterpillars feeding on control plants. Additionally, mortality of lacewings increased when they were provided with a diet of *B. philenor* caterpillars reared on supplemented leaves compared to caterpillars reared on control leaves. Thus, the induction of aristolochic acids in the plant following leaf damage does not resolve the problem confronted by the plant and may confer benefits to this sequestering specialist.

Introduction

Plants invest in an array of defensive strategies against herbivores. These can include structural defenses, such as thorns and trichomes (Ågren & Schemske, 1993), mechanical defenses that inhibit herbivore feeding, such as latex (Zalucki & Brower, 1992; Dussourd, 1997; Zalucki & Malcolm, 1999), and chemical defenses, such as toxins and inhibitors of digestion (Feeny, 1976; Berenbaum, 1995; Hammerschmidt & Schultz, 1996). As a result, many herbivorous insects are restricted to a narrow range of acceptable host plants to which they have evolved resistance or tolerance (Barbosa, 1988; Jaenike, 1990; Cohen et al., 1992). Many extreme specialists on toxic plant families sequester these toxins and in turn use them for their own defense against higher trophic levels (Price et al., 1980; Malcolm, 1992; Rowell-Rahier & Pasteels, 1992). This presents a problem for plants because their chemical defense actually confers fitness benefits on their herbivores and can increase herbivore numbers. The use of plant toxins by herbivores as a defense against higher trophic levels has been termed the lethal plant defense paradox and it is not known if plants have evolved strategies to resolve this problem (Price et al., 1980; Malcolm & Zalucki, 1996).

It has been demonstrated for an increasing number of systems that plants are not passive participants in

their interactions with herbivores and that defenses can increase following herbivore damage (Karban & Baldwin, 1997). Herbivore-induced defenses have been shown to be an effective adaptation for deterring later herbivory (Agrawal, 1998; Baldwin, 1998), attracting herbivore natural enemies (Dicke et al., 1993; Turlings et al., 1993, 1995; Havill & Raffa, 2000), and increasing the susceptibility of herbivores to parasite attack (Thaler, 1999). However, herbivore-induced responses can also have a detrimental effect on the plant by increasing its susceptibility to some herbivores (Messina et al., 1993) or by decreasing the virulence of some herbivore pathogens and parasites (Hunter & Schultz, 1993; Leather & Walsh, 1993). Induced responses can be effective against some specialist herbivores (Zalucki & Brower, 1992; Zangerl & Berenbaum, 1993; Osier et al., 1996). Little is known, however, about the effect of induced secondary compounds on sequestering specialists.

Malcolm & Zalucki (1996) suggested that a rapid induction followed by a net decrease of chemical defenses in plants might resolve the lethal plant defense paradox. Specifically, they reasoned that a rapid increase in plant toxins can be costly to newly hatched caterpillars due to the physiological cost of dealing with these compounds and that a later relaxation in these defenses would decrease the toxins available for sequestration. Thus, sequestering specialists may have to deal with the cost associated with increased plant toxins, but the plant withholds this resource as they begin to sequester defenses. Alternatively, however, if induced toxins have no negative effects on sequestering caterpillars, the problem confronted by the plant may be magnified by further increasing the chemical defense of its herbivores. Thus, for rapid induction to be an effective means for the plant to resolve the problem posed by sequestering specialist, the induced toxin must be an effective defense against early instar caterpillars and not provide a defensive benefit for the caterpillars against predators.

I tested whether rapid induction of sequesterable toxins can resolve the problem confronted by a plant with a sequestering specialist using the California population of the pipevine swallowtail, *Battus philenor* (L.). The California population is disjunct from the rest of the range of the species and is sometimes designated as the sub-species *B. philenor hirsuta* Skinner. Pipevine swallowtails are extreme specialists on plants in the genus *Aristolochia* (Racheli & Pariset, 1992) which are known to contain toxic alkaloids called aristolochic acids. Although these toxins are effective at deterring many generalist herbivores (Chen & Zhu, 1987; Park et al., 1997), the caterpillars of *B. philenor* sequester these toxins and, in turn, caterpillars and adult butterflies are chemically defended from predators (Brower, 1958; Rothschild et al., 1970; Fordyce, 2000). In California, only one species of *Aristolochia* is available, *Aristolochia californica* (Torrey), an endemic to the Central Valley, inner Coast Range, and foothills of the Sierra Nevada Mountians. The butterfly is the only herbivore commonly seen feeding on the plant. Little is known about the sequestration biology of these butterflies, particularly whether there is a cost associated with feeding on plants with aristolochic acid (Urzua et al., 1987). The first instar is vulnerable to attack by a number of predators (J.A. Fordyce and A.A. Agrawal, unpubl.). However, later instars are less susceptible to predators because of behavioral responses to predators, which includes violent thrashing and the eversion of osmeteria (Stamp, 1986) and, presumably, the accumulation of plant-derived chemical defense. The aim of this study was to determine if aristolochic acids are inducible in *A. californica*, if aristolochic acids are costly to newly hatched *B. philenor* caterpillars, and if the aristolochic acids of the host plant confer defensive benefits against caterpillar natural enemies.

Materials and methods

Inducibility of aristolochic acids in A. californica. To determine if aristolochic acids are induced in *A. californica* following plant damage I conducted an experiment in Stebbins Cold Canyon Ecological Reserve (Solano Co. CA). Twelve plants were chosen at random along a 1.5 km stretch of riparian habitat. The second most distal leaf was removed and placed in a Whirl Pak® on dry ice for a time-zero measure of aristolochic acid content. Groups of two, twelve, or zero neonate caterpillars were placed on the most distal leaf and permitted to feed. Plants were enclosed in spun polyester mesh bags (Kleen Test Products, Brown Deer, WI) to exclude natural enemies of the caterpillars. The most distal leaf was then collected from a randomly chosen subset of each herbivore treatment after 1, 2, 4, or 6 days and similarly placed in Whirl-Paks® on dry ice. All leaf material was stored in a −80°C freezer until chemical analysis. The experiment was repeated three times to control for any abiotic or phenological factors that may influence plant secondary chemistry. Leaf tissue was dried

under reduced pressure. Aristolochic acids were extracted from dried powdered leaf material sonicated in chloroform for 30 min using the method described in Fordyce (2000). The chloroform extract was dried under nitrogen and resuspended in 1 ml chloroform and transfered to an auto-sampler vial. Aristolochic acid was quantified using HPLC. Each injection was 20 μl injection eluted isocratically with a mixture of methanol-water-acetic acid (66:33:1) at 1 ml min^{-1} on a 250-4 LiChroCART RP-18 column packed with 5 μm LiChrospher 100 (E. Merck) with detection by photodiode array at 249 nm (Fordyce, 2000). Aristolochic acid (AA) was measured as μg AA mg^{-1} of leaf dry weight. Induction of aristolochic acid was described as the difference in aristolochic acid concentration between the time zero leaf and the later collected leaf on the same plant.

Effect of aristolochic acids on caterpillar preference. To assess if aristolochic acids affect caterpillar preference I carried out a choice test. Single leaves of *A. californica* (30 replicates) were placed in plastic petri dishes (145 mm diameter) with their petioles in 1.5 ml centrifuge tubes containing water to maintain leaf turgor. A mixture of aristolochic acid I and II obtained from Sigma was supplemented to one half of the leaf by applying a 50 μl of a 14% (w/v) solution of aristolochic acid in etOH using a 50 μl pipette. Aristolochic acids I and II are the primary aristolochic acids found in *A. californica* (Fordyce, 2000). The other half of the leaf received 50 μl 100% etOH as a control. Application was done outside in full sunlight and the etOH evaporated before leaf tissue damage occurred. The side of the leaf to receive each treatment was randomly determined using a coin toss. Supplementing the aristolochic acid content of the leaf results in a threefold increase in concentration compared to constitutive levels observed in the field (Fordyce, 2000). A single neonate caterpillar from wild-collected eggs was placed on the petiole adjacent to the leaf blade and permitted to feed for 48 hours. Leaves were then digitized and leaf area removed on either side of the leaf was determined using NIH image 1.62 software. Percent leaf material removed from either side was compared using a paired t test (arcsin-transformed) to assess if aristolochic acid concentration affected caterpillar preference.

Effect of aristolochic acid on caterpillar performance. Caterpillar performance and physiological cost were assessed as weight gain (μg). Four separate laboratory experiments were carried out to determine if aristolochic acids confer a physiological cost on developing caterpillars. All caterpillars were hatched from wild-collected eggs. (*Experiment 1*) One-half of an *A. californica* leaf was supplemented with 7 μg of aristolochic acid in an etOH solution as decribed above. The other half of the leaf received an equal amount of etOH as a control. The petiole of each leaf was placed in a 1.5 ml centrifuge tubes filled with water to maintain leave turgor. A single caterpillar was confined to a clip cage on either side of a leaf and permitted to feed for 24 hours. The experiment had 14 replicates. Caterpillars were weighed and the leaf was digitized to determine leaf area removed for both treatments. A paired t test on caterpillar weight was used to assess caterpillar performance. Caterpillar weight for each treatment was correlated with leaf area removed to assess if a physiological cost was associated with increased aristolochic acid content (i.e., treatment effect or interaction between treatment and leaf area consumed on caterpillar weight gain analyzed by ANCOVA). (*Experiment 2*) Twenty pairs of caterpillars were set up in an experiment as described above. Here the amount of aristolochic acid supplemented was 12 μg, resulting in nearly six times greater concentration than naturally occurs in *A. californica* leaf tissue. Caterpillars were weighed after 48 hours of feeding. (*Experiment 3*) To assess if increased leaf toxicity affects later stages of development, 120 caterpillars were placed on separate sprigs of *A. californica*. Sixty sprigs were supplemented with aristolochic acid in an etOH solution applied using a spray bottle. Application via this method results in an increase of approximately 0.438 μg of aristolochic acid per mg of leaf material dry weight. This resulted in leaf tissue with a sevenfold greater concentration of aristolochic acid than occurs naturally. The remaining sixty sprigs were sprayed with 100% etOH as a control. Fresh plant material received these treatments and was provided to the caterpillars every three days. Caterpillars were weighed and instar was recorded after six days of feeding and the butterflies were again weighed at pupation. Weights of caterpillars and pupae from these treatments were compared using an unpaired *t* test. (*Experiment 4*) To determine if a threshold for toxicity could be detected, a gradient of aristolochic acid was applied to leaves. Leaf squares (1.6 cm^2) were placed in petri dishes on damp filter paper. Each leaf square received 50 μl of an ethanol solution ranging from 0 to 12.3 μg aristolochic acid, in increments of 0.49 μg, giving a range of aristolochic acid that

exceeded seventeen times the natural concentration. Three replicates at each concentration gave 78 independent observations. Caterpillars were weighed after 48 hours of feeding.

Effect of caterpillar food plant toxicity on predators. I used lacewing (*Chrysoperla* spp.) larvae obtained from A-1 Unique Insect Control (Citrus Heights, CA) as a model predator. Two experiments were carried out to assess the effect of caterpillar hostplant toxicity on feeding and survival of lacewing larvae. Lacewings are a commonly observed predator of first instar *B. philenor* in the field (J. A. Fordyce, unpubl.). Lacewing larvae were raised on a diet of aphids (collected from rose bushes) until they molted into the ultimate instar. (*Experiment 1*) Sixteen lacewing larvae were starved for two days and placed in separate empty petri dishes. Each of these predators were fed one two-day-old first instar *B. philenor* caterpillars hatched from wild collected eggs. Eight of the caterpillars had been feeding on leaf material that was supplemented with 12 μg of aristolochic acid; the other eight were reared on control plants. Caterpillars were weighed before the experiment to control for differences in weight. Using soft-grip forceps, one caterpillar was placed on the mandibles of each lacewing and each lacewing was permitted to consume the caterpillar. All caterpillars were consumed. Five neonate *B. philenor* caterpillars were then placed in each arena. For the following 270 min each arena was monitored every 30 min to assess the capture success and feeding rate of each predator. Repeated measures ANOVA was used to compare the number of caterpillars remaining after each time interval between arenas with predators that had previously fed on caterpillars reared on aristolochic acid-supplemented diet vs. control diet. The mortality data of the neonates from each arena were also analyzed using Kaplan-Meier survival analysis to assess experimental cohort survival. (*Experiment 2*) Thirty-two ultimate instar lacewings were fed each day a diet of two *B. philenor* caterpillars hatched from wild collected eggs. Half of the lacewings received caterpillars that were feeding for two days on plants supplemented with six times the naturally occuring concentrations of aristolochic acid. The other half received control caterpillars of the same age reared on leaves with natural concentrations of aristolochic acid. The effect of caterpillar diet on predator mortality was monitored for both treatments.

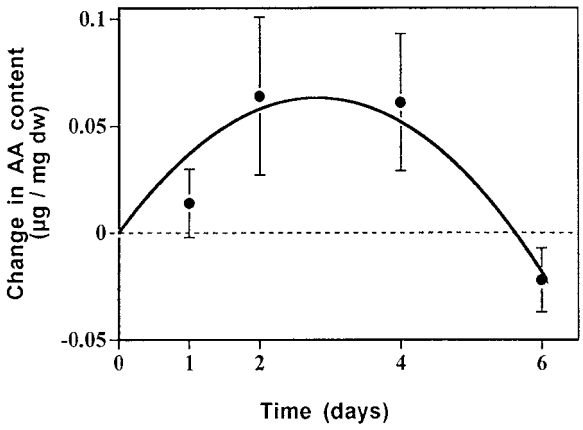

Figure 1. The change in aristolochic acid content (μg mg^{-1} of leaf dry weight) over time following initial damage. Error bars are standard errors of mean change in aristolochic acid content. Change in aristolochic acid = 0 + 0.045(days) −0.008(days)2 ($F_{2,29} = 5.836$, $P < 0.01$, $R^2 = 0.30$). Mean (± s.e.) constitutive aristolochic acid concentration is 0.062 (±0.014) μg mg^{-1} dw.

Results

Inducibility of aristolochic acids. Six samples were excluded from this analysis because they either had no remaining leaf tissue to analyze or they were damaged in the field, presumably by mammals attracted to the plant sleeves. Groups of twelve larvae generally consumed between 50% and 100% of the apical leaf. Groups of two larvae consumed less than 25% of the apical leaf. Initially group size was manipulated to determine if various herbivore densities influence the induction of aristolochic acids; however, ANOVA showed no significant differences among the herbivore treatments for any day [day(F); 1(1.635), 2(0.269), 4(0.141), 6(0.782)]. Because I was interested in characterizing the induction in response to plant damage, and no effect of subsequent herbivore group size was detected, I pooled all of the treatments to characterize change in aristolochic acid from time zero to the time the second leaf was harvested. Aristolochic acids were induced following the removal of the time zero leaf (Figure 1). Aristolochic acid concentration of the leaves was double that of constitutive concentrations two and four days following initial damage to the plant. After six days, however, aristolochic acid content returned to that of undamaged leaves.

Effect of aristolochic acid on caterpillar preference and performance. Seven of the thirty replicate caterpillars in the choice test crawled into the centrifuge tube and died, leaving 23 replicates. The amount of

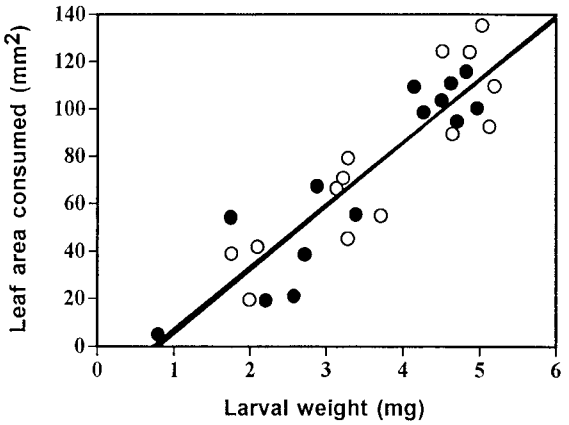

Figure 2. Correlation between caterpillar weight and leaf area consumed. Solid circles indicate control ($r = 0.92$, $P < 0.0001$). Open circles indicate supplemented aristolochic acid treatment ($r = 0.88$, $P < 0.0001$). ANCOVA revealed no physiological cost for caterpillars consuming leaves with three times the natural quantity of aristolochic acid (i.e., no detectable treatment effect or interaction between treatment and leaf area consumed on weight gain).

leaf material consumed did not differ significantly between portions of the leaf with supplemented aristolochic acid and the control portion ($t = 0.927$, $df = 22$, $P = 0.364$). Thus, the aristolochic acid content of the leaves did not affect caterpillar feeding preference.

Caterpillar growth after 24 and 48 hours was not influenced by the presence of supplemented aristolochic acid (Table 1). As may be expected, caterpillar weight after 24 hours of feeding was correlated with the amount of leaf material consumed for caterpillars feeding on leaves with supplemented aristolochic acid and control leaves (Figure 2). However, ANCOVA did not detect an interaction between aristolochic acid content and caterpillar weight ($F_{1,24} = 0.057$, $P = 0.814$). Thus, no additional physiological cost was detected for caterpillars feeding on leaves with supplemented aristolochic acid compared to caterpillars feeding on un-supplemented leaves.

Weights of caterpillars feeding on sprigs of *A. californica* supplemented with aristolochic acid did not differ after six days of feeding compared to control caterpillars feeding on leaves without supplemented aristolochic acid (Table 1). Furthermore, there was no difference in mortality between treatments ($\chi^2 = 1.37$, $df = 1$, Fisher's exact test $P = 0.243$) or the number of caterpillars that had successfully molted into the second or third instar ($\chi^2 = 2.16$, $df = 2$, $P = 0.340$). Similarly, pupal weights did not differ between the two treatments (Table 1), and caterpillar mortality prior to pupation was the same for both treatments ($\chi^2 = 1.00$, $df = 1$, Fisher's exact test $P = 0.316$). A gradient of aristolochic acid supplementation ranging from natural levels to more than 17 times levels encountered in nature failed to reveal a threshold for aristolochic acid toxicity, measured as weight gain, after 48 hours of feeding by neonate caterpillars ($F_{1,55} = 0.038$, $P = 0.846$).

Effect of caterpillar food plant toxicity on predators. The weights of the caterpillars fed to the lacewings were not different between caterpillars reared on supplemented and unsupplemented leaves ($t = 0.234$, $df = 28$, $P = 0.816$). Thus, the weight of the initial caterpillar meal can be discarded as a confounding factor influencing subsequent predator feeding rate. Predators that had fed on a single caterpillar raised on plants with supplemented aristolochic acid subsequently found and consumed other caterpillars at a reduced rate (Table 2; Figure 3a). Neonates in arenas with predators that had fed initially on caterpillars with supplemented aristolochic acid were more likely to survive over the course of the experiment (Figure 3b). More than 80% of the arenas with predators who had fed upon supplemented caterpillars had remaining neonates at the end of the experiment compared to fewer than 30% for predators who had fed on control caterpillars. No caterpillars remained in any of the arenas 24 hours after the beginning of the experiment.

The amount of aristolochic acid available to *B. philenor* had an effect on predatory lacewing survival ($\chi^2 = 4.63$, $df = 1$, $P < 0.05$). Nearly 65% of lacewings feeding on butterfly caterpillars reared on leaf material with supplemented aristolochic acid died before metamorphosing. Only 27% of the lacewings feeding on control caterpillars died. Thus, the toxicity of the butterfly food plant had a negative effect on lacewing survival.

Discussion

Aristolochic acids were induced in *A. californica* following plant damage. The induction observed in this study was likely due to the removal of a leaf at the start of the experiment, as no effect of subsequent herbivory was detected. Mechanical damage of leaves has similarly been shown to induce chemical defenses in other plant species (Karban & Baldwin, 1997). A different experimental design will have to be used to determine whether this induced response varies with different

Table 1. Comparisons of mean weights of *B. philenor* feeding on control leaves and leaves supplemented with aristolochic acid (AA)

Experiment	Food	*n*	Mean weight mg (± s.e.)	*t*
(1) Caterpillars (24 h feeding)	Control	13	3.45 (*0.35*)	1.02 (NS)[a]
	AA Suppl.	13	3.71 (*0.32*)	
(2) Caterpillars (48 h feeding)	Control	20	5.00 (*0.24*)	0.76 (NS)[a]
	AA Suppl.	20	5.23 (*0.18*)	
(3) Caterpillars (144 h feeding)	Control	57	35.38 (*1.40*)	0.02 (NS)[b]
	AA Suppl.	58	35.43 (*1.56*)	
(4) Pupae	Control	29	1241 (*44*)	1.42 (NS)[b]
	AA Suppl.	24	1174 (*28*)	

[a] Paired *t*-test.
[b] Unpaired *t*-test.

Table 2. Repeated measures ANOVA for number of remaining neonate caterpillars in each arena with predators that fed on an initial meal of a two day old caterpillar reared on control plants or plants supplemented with 12 μg aristolochic acid

Source	df	MS	*F*	P
Caterpillar food	1	41.863	4.172	0.0619
Error	13	10.033		
Time	9	23.155	34.101	<0.0001
Time × caterpillar food	9	2.029	2.988	0.0031
Error	117	79.445		

densities of *B. philenor* caterpillars. The shape of the aristolochic acid induction curve described for *A. californica* is not surprising. Many other investigators have similarly described an initial increase after the plant is damaged followed by a decrease below constitutive levels of toxins (Malcolm & Zalucki, 1996; Zangerl & Berenbaum, 1995; McCloud et al., 1995). However, based upon the performance experiments conducted in this study, it is unlikely that these rapidly induced toxins play a defensive role against *B. philenor* caterpillars.

Although inducible defenses have received a great deal of attention in the past twenty years, it is highly unlikely that these responses are as novel for specialist herbivores as they are for investigators. Indeed numerous examples have been described for herbivores that modify feeding behaviour in anticipation of induced plant responses (McCloud et al., 1995; Dussourd & Eisner, 1987; Dussourd, 1997). For inducible responses to have any effect on specialist herbivores there must be a cost incurred for the herbivore when dealing with these defenses (Karban & Baldwin, 1997). I found no evidence that aristolochic acids at concentrations much greater than would ever be encountered in *A. californica* have any effect on *B. philenor* growth, preference, or survival. Aristolochic acids are toxic to many herbivores (Park et al., 1997); however, specialists on these plants must have some physiological mechanism to deal with these toxins. No cost associated with these toxins was detected for *B. philenor* in this study. This is in contrast to specialists that feed on other toxic plant families. For example, Zalucki & Brower (1992) detected a cost for early instar monarch caterpillars in relation to the cardenolide and latex defenses of their *Asclepias* hostplants. Rapid induction of cardenolide defenses thus may play an important defensive function for defense against these specialist herbivores (Malcolm & Zalucki, 1996). However, it does not appear that a similar argument can be made for the efficacy of inducible aristolochic acids in *A. californica* against *B. philenor*.

An increase in aristolochic acid following plant wounding may supply *B. philenor* caterpillars with an important resource that they sequester. Thus, induction of aristolochic acids does not resolve the lethal plant defense paradox, rather it may magnify the problem for the plant. Increased toxin content apparently increases herbivore defense against at least one common natural enemy. Caterpillars feeding on leaf tissue supplemented with additional aristolochic acid had a greater negative effect on lacewing predators when compared to caterpillars feeding on un-supplemented control plants. This is the first direct experimental link between *B. philenor* defense and the aristolochic acid content of its hostplant. Although the arena ex-

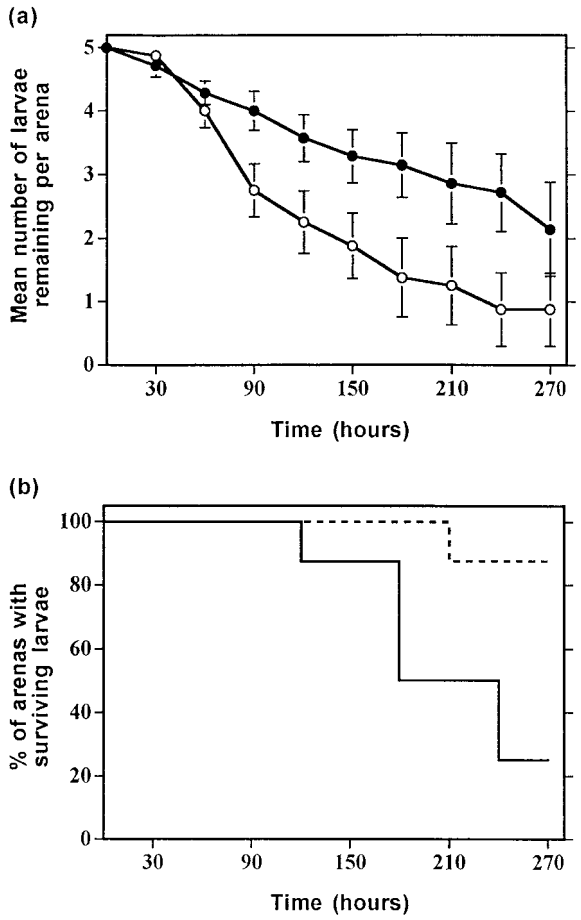

Figure 3. The effect of *B. philenor* caterpillar food plant toxicity on foraging and feeding of lacewing larvae. (a) The mean number of caterpillars remaining in each arena. Error bars denote standard error. Open circles are arenas with predator that initially fed upon control caterpillars. Closed circles are arenas with predators that initially fed on caterpillars reared on leaves with supplemented aristolochic acid. (b) Survival plot for neonate cohorts placed in each arena. Solid line is control group. Dashed line is arenas with predators that initially fed upon caterpillars with an aristolochic acid supplemented diet. Caterpillar cohorts in arenas with predators that had consumed a caterpillar reared on supplemented aristolochic acid diet were more likely to have some remaining members by the end of the experiment (Mantel–Cox $\chi^2 = 5.73$, df $= 1$, P $= 0.02$).

periment was artificial, in that caterpillars could not escape from the predator, some individuals from each caterpillar cohort were more likely to survive if the predator had consumed a caterpillar reared on leaves with increased toxins. If toxic caterpillars influence the foraging ability and capture rate of predators, they may be able to escape predators in their natural environment. In California, young *B. philenor* caterpillars feed in tight aggregations of related individuals. Disturbing a single individual in these tight aggregations often causes the group to disperse, often to another leaf on the plant, and then to re-group, usually in smaller groups, and continue feeding in an area separate from the initial disturbance (pers. obs.). Because first instar caterpillars are most susceptible to predation, the toxic effect experienced by the predator may increase the time available to caterpillars to feed and subsequently molt to the less vulnerable second instar.

It is likely that aristolochic acids are but one defense against herbivory found in *A. californica*. Recently the presence of protease inhibitors has been confirmed for this plant (pers. obs.). It is not known if induction of these or other plant defenses are effective at deterring herbivory by *B. philenor* caterpillars. However, the lethal plant defense paradox remains for *A. californica*, as their sequestering specialists reap the benefits of aristolochic acids but incur no detectable cost.

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