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Slow movement increases the survivorship of a chemically defended grasshopper in predatory encounters

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Abstract Studies on insect defenses have investigated the benefits of noxious chemicals, aposematic coloration, and even gregariousness, but little information exists on the role of slow movement (as opposed to rapid escape movement and the absence of movement). Using the chemically defended, slow-moving lubber grasshopper (*Romalea guttata*) and the northern leopard frog (*Rana pipiens*), we investigated the role of slow movement in insect defense. In a five-day experiment, frogs did not learn an aversion to lettuce-fed lubbers; we infer that endogenous deterrent chemicals are not important in lubber defense from frogs. In experiment 2, lubbers moved significantly more slowly than crickets in the presence of frogs held under beakers. In experiment 3, control (i.e., slow-moving) lubbers suffered significantly less predation than motion-induced lubbers, and frogs attacked control lubbers significantly later than they attacked motion-induced lubbers. Hence, slow movement appears to be an important component in enhancing lubber survivorship in frog encounters. This is the first demonstration that the slow movement of an aposematic insect increases its survival by failing to release the attack response of certain motion-oriented predators.

Key words Slow movement · Lubber grasshopper · Chemical defense syndrome · Northern leopard frog · Prey movement

Introduction

Insect defenses have probably evolved because of selection pressures from vertebrate predators (Evans and

Schmidt 1990). Many unpalatable insects exhibit the suite of characters known as the chemical defense syndrome (CDS); these insects are often aposematic, gregarious, and slow moving (Whitman et al. 1985). For these characteristics to have evolved and persist, each one should contribute to the fitness of extant insects (Hatle and Townsend 1996). Many previous studies have investigated the benefits of noxious chemicals (Whitman et al. 1990) and suggest that predators may either release (Wiklund and Järvi 1982) or learn an aversion to (Coppinger 1970; Evans and Schmidt 1990) chemically defended insects. Some insects may even adapt the composition of defensive secretions in response to local predators (Steidle and Dettner 1993). Aposematic coloration has been shown to be advantageous to insects in encounters with birds (Järvi et al. 1981; Marples et al. 1994) and lizards (Johki and Hidaka 1979). Bright prey colors may innately deter predators (Guilford 1988), and/or they may facilitate aversion learning (Roper and Wistow 1986). Gregariousness has recently been shown to be beneficial in concert with unpalatability (Alatalo and Mappes 1996).

In contrast, little information exists on the benefits of slow movement for insect defense (Evans and Schmidt 1990). Some insects use rapid movements to escape predators (Camhi 1980), while others remain motionless to avoid detection by predators (Tarsitano and Jackson 1992). Slow movement, particularly in aposematic insects, is distinct from both of these behaviors, because the insect is neither (1) running away from nor (2) apparently avoiding visual detection by the predator. In this paper, we investigate the role of slow movement in the defense of a chemically defended insect from vertebrate predators.

Juveniles of the eastern lubber grasshopper, *Romalea guttata* (= *microptera* Houttuyn), are chemically defended, aposematic, gregarious, and slow moving (Whitman 1988). In the wild, these black and yellow grasshoppers are often found in plain sight and are easily caught by hand. A single researcher can catch 100 lubbers in about 15 min (J.D. Hatle, personal observa-

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tion). Lubbers are effectively defended from most birds (Whitman 1988) and anoles (Hatle and Townsend 1996, in press), but perhaps by different mechanisms. Feeding experiments have shown that naive starlings (*Sturnus vulgaris*) attack first-instar lubbers and then spit them out or vomit; experienced starlings refuse offerings of lubbers. Whitman (1988) concluded that starlings appear to be deterred by repugnant compounds stored inside lubbers. In contrast to avian predators, naive lizards (*Anolis carolinensis*) failed to attack juvenile lubbers (Hatle and Townsend 1996). Thus, anoles appear to be deterred before attack rather than by internal repugnant compounds which depend on tasting to be effective.

In southern Louisiana, lubbers are sympatric with several species of frogs in the genus *Rana* [e.g., *R. utricularia* (= *sphenocephala*), the southern leopard frog; *R. clamitans*, the bronze frog; *R. catesbeiana*, the bullfrog; Conant and Collins 1991]. The northern leopard frog, *R. pipiens* (Schreber), does not co-occur with the eastern lubber grasshopper. We used *R. pipiens* as a model predator because it is allopatric with lubber grasshoppers and is thus a lubber-naive assay for the rapid species that are sympatric with lubbers. Predator naivety is essential in experiments testing for learned aversions to chemically defended prey. *R. pipiens* is a sit-and-wait, motion-oriented predator often used to study predation (e.g., Anderson 1993). Northern leopard frogs can use olfactory cues to distinguish between visually identical prey items (Sternthal 1974) and can learn to avoid noxious prey (Rice and Taylor 1995).

Our central hypothesis was that slow movement by unpalatable insects is an effective defense against motion-oriented predators (Whitman 1988; Hatle and Townsend 1996). To test this hypothesis, we fed lubbers a diet low in allelochemicals. In this way, we were able to produce an insect that was not chemically defended but still exhibited its characteristic slow-movement behavior. We then manipulated the motion of the insect to determine the role of movement in its defense. We conducted three experiments, each addressing a single question, to test our central hypothesis. First, is lubber survivorship attributable to endogenous deterrent chemicals (i.e., do northern leopard frogs learn an aversion to lettuce-fed lubbers)? Second, do lubbers in the presence of frogs move more slowly than either crickets in the presence of frogs or lubbers in the absence of predators? Third, does slow movement increase the survivorship of lubbers in feeding trials with frogs?

Materials and methods

Experiment 1: Is lubber survivorship attributable to endogenous deterrent chemicals?

Experimental animals

Lubbers were hatched in the laboratory of Dr. D.W. Whitman (Illinois State University, USA). They were fed a mixed diet of

romaine lettuce, green beans, green onion, and apple (Whitman 1986) until they were second instars. They were then shipped to our laboratory (University of Southwestern Louisiana, USA) and maintained on lettuce and cricket chow. These lubbers had essentially no allelochemicals in their diets, and any chemical defenses they possessed were endogenous. We purchased adult crickets (*Acheta domesticus* L.) from Fluker's Cricket Farm (Baton Rouge, La., USA) and fed them Purina Cricket Chow. All insects were kept at 30°C until immediately before the experiment. We purchased 17 northern leopard frogs from Carolina Biological Supply Co. (Burlington, N. C., USA) and held them together at 21 ± 2°C until two days before the experiments. During this time, we fed each frog three adult crickets, twice weekly. Before the experiments, we transferred all frogs to individual plastic containers (30 cm × 30 cm × 10 cm) containing 250 ml tap water and 150 ml pea gravel, at 26 ± 2°C. The final pre-experimental feeding (in the frog's home cage) was followed by two days in which the frogs were denied food; in this way, frogs were maintained on the same feeding schedule throughout the experiment. This method ensured that the northern leopard frogs were initially naive to lubbers, but not to crickets.

Feeding trials

We conducted trials on five feeding days; consecutive feeding days were separated by two days in which the frogs were not fed. On each feeding day, in a random order, we offered each frog three prey items of a similar size. Specifically, each frog was sequentially offered an adult cricket (cricket I), a 3rd- or 4th-instar lubber, and finally a second adult cricket (cricket II). Each feeding trial was identical, except for the prey item offered. The crickets were used as a positive control, to determine whether the frogs were ready to feed. We began trials by introducing an insect into the frog's cage and measured: (1) the time of the frog's first orientation toward the prey item (= orientation), and (2) the time of the frog's first snap at the prey item (= snap). Orientation and snapping are sequential measures of the anuran predation response (Ewert 1987). Each trial ended when the prey item's torso was completely inside the frog's mouth (= eaten) or at the arbitrary time limit of 300 s, whichever occurred first. We recorded insect survival at the end of the trial and began the next trial about 2 min later.

Statistical analyses

We analyzed the insect survival data using a categorical model for repeated trials (SAS 1990). This weighted-least-squares model an-

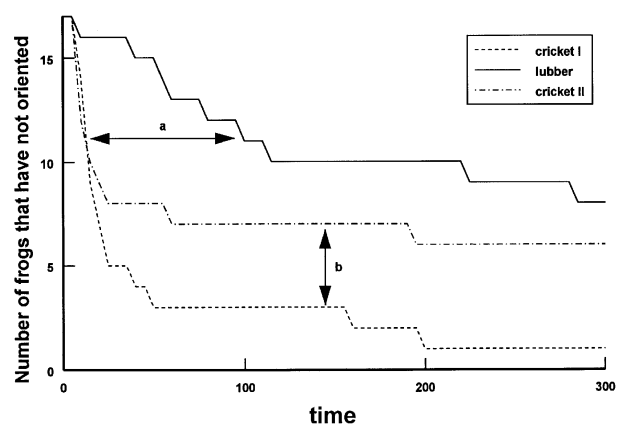


Fig. 1 Example of response-time curves using orientation data for day 1. Curves may be significantly different because of response times (arrow a) or number of individuals responding (arrow b)

alyzed the goodness of fit of our data to a linear model. The number of insects that survived was analyzed for effect of prey item (i.e., among cricket I, lubber, and cricket II), effect of feeding day, and the interaction between prey item and feeding day. The test for interaction between prey item (i.e., lubber) and feeding day was most important because it revealed whether the frogs learned an aversion to the chemical defenses of lubbers.

We analyzed predator time until first orientation and first snap with the Kaplan-Meier time-failure analysis, which is appropriate for time-dependent data (Lee 1980). For each possible combination of feeding day and prey item, we generated a response-time estimate curve (Fig. 1) and compared the curves using pairwise tests (White and Garrolt 1990). This comparison weighs both the time of each response (e.g., frog 1 first oriented to the lubber at 79 s) and the number of responses (e.g., 9 of 17 frogs oriented towards lubbers). In these analyses, therefore, both the median time of response and the number of frogs responding were important. Orientations to and snaps at lubbers on each feeding day were tested against orientations to and snaps at lubbers on all other feeding days. Because we used ten pairwise comparisons for each behavior, $\alpha = 0.005$ (Bonferroni's adjustment).

Experiment 2: Do lubbers in the presence of frogs move more slowly than either crickets in the presence of frogs or lubbers in the absence of predators?

Experimental animals

Insects were obtained and maintained exactly as in experiment 1. We obtained northern leopard frogs from Wm. A. Lemberger Co. (Oshkosh, Wisc., USA) and held them together at $21 \pm 2^\circ\text{C}$ until immediately before the experiment.

Rate-of-movement trials

We conducted these experiments in a 22.3-l plastic tub with a 14×9 grid on the entire bottom. Each grid was 2.5 cm^2 . We painted the bottom of the inside walls of the tub with Fluon AD-1 (Northern Products, Woonsocket, R.I., USA), an opaque Teflon paint that deterred the insects from climbing the walls of the tub. We estimated rate of movement by counting the number of times the insect's right front tarsus crossed a gridline during 180-s trials. In experiments 2a and 2b, we tested the rate of movement of 11 3rd-instar lubbers and 11 adult crickets. We placed a "predator" in the tub between the center of the floor and the wall. Each trial was started by releasing an insect at the center of the floor facing away from the "predator." In experiment 2a, the insects were tested in the absence of predators. In experiment 2b, the "predator" was a live frog under a 600-ml clear Pyrex beaker. We did not standardize the direction in which the frogs were facing at the start of a trial. The pour spout of the beaker allowed passage of gases between the frog and the insect. To deter insect climbing, we painted a ring of Fluon on the outside of the beaker. The Fluon was painted at a height that we believed the insects could see under and the frogs could see over. During the trials, several frogs oriented toward and even snapped at lubbers, implying that they actually could see the lubbers. We used the same insects in experiments 2a and 2b. We calculated the total number of times each insect crossed a gridline during the 180-s trials. These sums were compared using two-tailed paired t-tests.

In experiment 2c, we compared the rate of movement of lubbers not in the presence of a predator to lubbers in the presence of a frog under a beaker. The methods were identical to experiment 2b with the following exceptions. All trials were 300 s. We tested all lubbers first without a predator, then with a frog predator under a beaker, and then again without a predator. We used a repeated-measures ANOVA to compare the data sets.

Experiment 3: does slow movement increase the survivorship of lubbers in feeding trials with frogs?

Experimental animals

For this experiment, we collected lubbers as 2nd and 3rd instars near Lydia, La., USA. We fed all lubbers romaine lettuce and cricket chow in the laboratory. The lubbers may have sequestered allomones in the wild, but because they were randomly distributed between the two treatment groups, these putative allomones should not have affected our results. All lubbers were acclimated to the experimental temperature ($26 \pm 2^\circ\text{C}$) before the trials and tested as 3rd or 4th instars. We trained 23 northern leopard frogs to eat lubbers in their home cages by feeding them lubbers on at least three separate days. With this exception, frogs were maintained identically to experiment 1. Eight of the 23 frogs used in experiment 3 had also participated in experiment 1.

Feeding trials

These experiments were conducted in a 20^{-1} aquarium instead of the frogs' home cages. To induce motion in the lubbers, we encircled their thoraxes with thread and tethered them from overhead. An electric motor was positioned adjacent to the attachment point of the thread. The lever arm of the motor plucked the thread about once a second, the frequency that Borchers et al. (1978) reported induced the maximal attack response in toads. This design jiggled the lubber about 1 cm up and back down to the original position. We termed these lubbers "fast moving." For control trials, we merely tethered lubbers, so that controls were identical to experimentals except that the lever arm of the motor did not pluck the thread and therefore did not move the lubber. These control lubbers were tethered loosely, so that they could move 2 or 3 cm across the floor of the aquarium on their own. This control tended to move lubbers more than they move on their own, because lubbers would occasionally reach the end of the tether and be pulled back to the center of the aquarium. We termed these control lubbers "slow moving." In this way, we presented palatable prey items that differed only in movement: slow-moving lubbers were essentially free to exhibit their natural behavior in the presence of frogs, but fast-moving lubbers were manipulated to jig at a regular interval. During the preparation of the prey item, the frog was acclimated under an opaque plastic container. We began trials by starting the electric motor and uncovering the frog. Trials lasted until the prey item was eaten or at the arbitrary time limit of 180 s, whichever occurred first. To minimize the frog's reactions to the experimenter, we observed the behavior of each animal through a small hole in a cardboard wall. We collected data as in experiment 1.

This experiment was counterbalanced in a two-day mixed design. On feeding day 1, we offered half of the frogs first a fast-moving lubber and (after covering the frog with the opaque plastic container but without removing the frog from the aquarium) then a slow-moving lubber. The remaining half of the frogs were presented prey items in the opposite order. On the next day, the frogs were denied food. On feeding day 2, we reversed the order of prey presentation for each frog.

Statistical analyses

We analyzed insect survival data by testing total fast-moving lubbers eaten by each frog vs. total slow-moving lubbers eaten by each frog. These paired, ordinal data were tested with a one-tailed Wilcoxon matched-pair signed-ranks test. To analyze orientation and snapping data, we assigned the maximum value of 180 s as the attack time for each frog that did not attack the prey item. We analyzed the data with one-tailed Wilcoxon matched-pairs signed-ranks tests. Because we used a paired rank test, the assignment of 180 s (as the value for frogs that did not attack the prey item) did not indicate an actual attack, but was merely a relative measure of the frog's behavior (or lack thereof) toward the prey item.

Results

Experiment 1: Is lubber survivorship attributable to endogenous deterrent chemicals?

Insect survivorship

The interaction between prey item and feeding day, indicating the changing survivorship of lubbers throughout the experiment, was not significant (Fig. 2). The non-significant trend was for the frogs to eat more lettuce-fed lubbers as the experiment progressed; this trend is important, because it is the opposite of what one would expect if the frogs were learning an aversion to lettuce-fed lubbers. All lettuce-fed lubbers that survived the feeding trials were still alive about 24 h later (i.e., no lubbers died after being snapped at and then released). Lettuce-fed lubbers (initially novel) survived the feeding trials with northern leopard frogs significantly more often than did crickets (initially familiar), which were eaten at a constant rate throughout the experiment. The survivorship of all three prey items combined was not statistically different over the five feeding days (i.e., the hunger of the frogs did not appear to differ among the five days). Frogs typically ate no more than two insects in a given day.

Frog responses to lubbers

The times of first orientation to lubbers decreased with the frogs' increasing familiarity with lubbers as prey items (Fig. 3). The response curves for orientations on day 1 were statistically different to those for orientations on days 2 ($\chi^2 = 39.8$; $P < 0.005$), 3 ($\chi^2 = 79.0$, $P < 0.005$), and 4 ($\chi^2 = 12.7$, $P < 0.005$). The response curves for orientations on day 1 and the orientations on day 5 were not statistically different ($\chi^2 = 0.019$, $P > 0.005$); this aberrant result was due to the number of frogs ultimately orienting to lubbers on the two days, in spite of the fact that the frogs oriented much later on day 1 than on day 5. The response curves for orientations on day 2 were statistically different to the response curves for orientation on days 4 ($\chi^2 = 78.0$, $P < 0.005$) and 5 ($\chi^2 = 28.5$, $P < 0.005$). The response curves for orientations on day 2 were not statistically different to the response curves for orientations on day 3 ($\chi^2 = 5.87$, $P > 0.005$). The response curves for orientations on day 3 were statistically different to the response curves for orientations on days 4 ($\chi^2 = 111$, $P < 0.005$) and 5 ($\chi^2 = 49.7$, $P < 0.005$). Finally, the response curves for orientations on day 4 were not statistically different than the response curves for orientations on day 5 ($\chi^2 = 3.09$, $P > 0.005$). In sum, orientation response curves for non-adjacent days were always statistically different with the single exception of day 1 versus day 5.

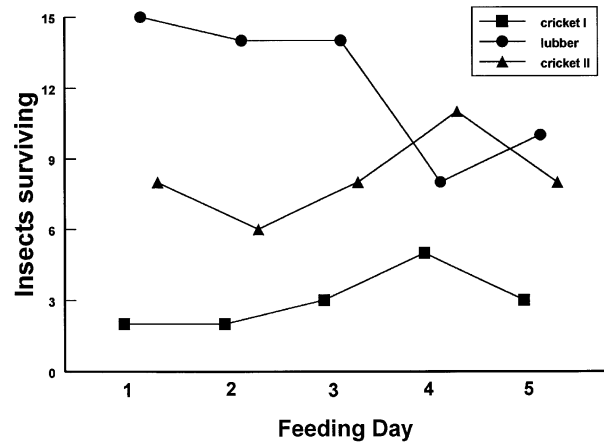


Fig. 2 Survivorship of lubbers and crickets in 300-s feeding trials with 17 northern leopard frogs. At the beginning of the experiment, the frogs were familiar with crickets (i.e., the crickets were a positive control) but naive to lubbers. We analyzed the data with a categorical model for repeated trials. The effect of prey item was significant ($\chi^2 = 44.3$, $df = 2$, $P < 0.001$), the effect of feeding day was not significant ($\chi^2 = 2.66$, $df = 4$, $P = 0.62$), and the interaction of prey item and feeding day was not significant ($\chi^2 = 15.0$, $df = 8$, $P = 0.059$)

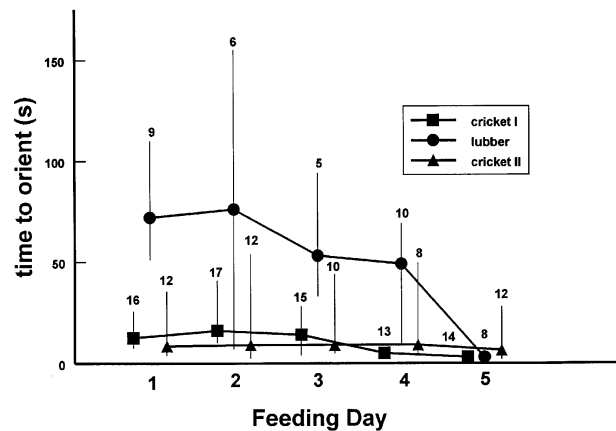


Fig. 3 Times to first orientation of northern leopard frogs to crickets and lubber grasshoppers in laboratory trials. At the beginning of the experiment, the frogs were familiar with crickets (i.e., the crickets were a positive control) but naive to lubbers. Each feeding day, each frog was offered first cricket I, next a lubber, and finally cricket II. Feeding days were separated by two days in which the frogs were denied food. Each group of data is represented by the median and the interquartile range; numbers above each group of data are the number of frogs (out of 17) that oriented to the prey item

The times of first snaps at lubbers decreased with the frogs' increasing familiarity with lubbers as prey items (Fig. 4). The response curves for snaps on day 1 were statistically different to those for snaps on days 2 ($\chi^2 = 78.2$, $P < 0.005$), 3 ($\chi^2 = 47.9$, $P < 0.005$), 4 ($\chi^2 = 104$, $P < 0.005$), and 5 ($\chi^2 = 17.5$, $P < 0.005$). The response curves for snaps on day 2 were statistically different to those on days 4 ($\chi^2 = 305$, $P < 0.005$), and 5 ($\chi^2 = 101$, $p < 0.005$). The response curves for snaps on day 2 were not statistically different to the response curves for snaps on day 3 ($\chi^2 = 0.156$, $P > 0.005$). The

response curves for snaps on day 3 were statistically different to those on days 4 ($\chi^2 = 213, P < 0.005$) and 5 ($\chi^2 = 88.9, P < 0.005$). The response curves for snaps on day 4 were statistically different to those on day 5 ($\chi^2 = 9.15, P < 0.005$). In sum, snapping response curves were always statistically different with the single exception of day 2 vs. day 3.

On the first feeding day, three frogs snapped at and then released or missed lubbers and subsequently failed to eat the lubber during that trial. (We did not attempt to distinguish release from miss). All three of these attacked lubbers survived for at least 24 h past the feeding trial. The same three frogs ate lubbers in at least two subsequent trials. Only one further instance of snap and release or miss behavior was observed during the study. This frog also ate a lubber in a subsequent trial.

Experiment 2: Do lubbers in the presence of frogs move more slowly than either crickets in the presence of frogs or lubbers in the absence of predators?

Lubbers moved significantly more slowly than crickets in the presence of either a plastic frog “predator” or a

live frog under a beaker (Table 1). Both lubbers and crickets moved at essentially constant rates throughout the 180 s trials, with the exception of the first 10-s interval, during which both species moved at least twice as fast as in the remainder of the trials. In comparisons across treatments, lubbers in the presence of a plastic frog did not move differently to lubbers in the presence of a live frog under a beaker ($t = 0.755, df = 20, P = 0.459$). Similarly, crickets in the presence of a plastic frog did not move differently to crickets in the presence of a live frog under a beaker ($t = 1.50, df = 20, p = 0.148$).

Lubbers in the absence of predators did not move significantly differently than lubbers in the presence of a frog under a beaker (Table 2). The non-significant trend was for lubbers to move more slowly in the presence of a frog under a beaker than in the absence of predators.

Experiment 3: Does slow movement increase the survivorship of lubbers in feeding trials with frogs?

Insect survivorship

Slow-moving lubbers had significantly higher survivorship than fast-moving lubbers [T – (sum of negative ranks) = 4.5, n' (non-zero scores) = 8, $P = 0.0273$]. The frogs ate a mean (\pm SE) of 0.652 (\pm 0.16) slow-moving lubbers and 0.913 \pm 0.19 fast-moving lubbers and a median of 0.0 slow-moving lubbers (IQR = 0–1) and 1.0 fast-moving lubbers (IQR = 0–2). One frog preferentially ate slow-moving lubbers whereas seven of the frogs preferentially ate fast-moving lubbers. We infer that prey movement significantly influenced insect survivorship.

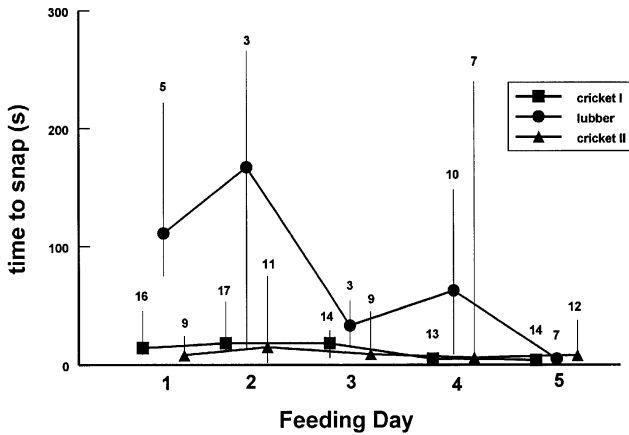


Fig. 4 Times of first snap by northern leopard frogs at crickets and lubber grasshoppers in laboratory trials. At the beginning of the experiment, the frogs were familiar with crickets (i.e., the crickets were a positive control) but naive to lubbers. Each feeding day, each frog was offered first cricket I, next a lubber, and finally cricket II. Feeding days were separated by two days in which the frogs were denied food. Each group of data is represented by the median and the interquartile range; numbers above each group of data are the number of frogs (out of 17) that snapped at the prey item

Table 2 Number of times per 300-s trial a lubber’s right front tarsus crossed a line on a grid composed of 2.5-cm² squares. The experiment was conducted in an ABA design with each lubber receiving all three treatments in series

	No predator	Frog under beaker	No predator
Mean \pm SE	44.5 \pm 7.35	24.5 \pm 4.69	48.5 \pm 10.2
F	2.78		
P	0.0841		
n	12		

Table 1 Number of times per 180-s trial an insect’s right front tarsus crossed a line on a grid composed of 2.5-cm² squares. The type of “predator” used in each experiment is presented in parentheses. For both experiments $n = 11$ insects and $df = 20$

	Experiment 1a (plastic frog)		Experiment 1b (live frog under beaker)	
	Lubbers	Crickets	Lubbers	Crickets
Mean \pm SE	21.0 \pm 7.30	108 \pm 14.1	15.0 \pm 3.16	78.5 \pm 13.2
Two-tailed t	5.46		4.69	
P	<0.0001		= 0.0001	

Frog responses

Northern leopard frogs oriented towards fast-moving lubbers significantly sooner than they oriented towards slow-moving lubbers ($T+$ (sum of positive ranks) = 22, $n' = 16$; $P = 0.0078$]; Fig. 5). Frogs snapped at fast-moving lubbers significantly sooner than they snapped at slow-moving lubbers ($T+ = 14$; $n' = 14$; $P = 0.0067$). We infer that prey motion significantly influenced frog predatory behavior.

Discussion

Our data suggest that slow movement by insects functions to deter predation. In our experiments, slow movement of prey increased the survivorship of lubber grasshoppers in trials with northern leopard frogs. These frogs were trained to eat lettuce-fed lubbers and had learned, presumably, that they were palatable. Nonetheless, the slow movement of lubbers increased not only the latent period before frog attacks but also the survivorship of the insects. We believe this is the first demonstration that the behavior of an aposematic slow-moving insect may help increase its survivorship in comparison to fast-moving insects.

Lubber defense does not appear to be due to endogenous deterrent chemicals

When lettuce-fed lubbers were novel prey, but crickets were not, our frogs ate more crickets than lubbers (Fig. 2) and attacked crickets sooner than lubbers (Figs. 3, 4). These data are not evidence that lubbers are defended from frogs; lubber novelty alone can explain these results. However, as lubbers became more familiar prey items, the frogs oriented towards and snapped at lubbers sooner (Figs. 3, 4). In other words, our frog response data imply that the frogs were learning that lettuce-fed lubbers were palatable prey.

The same story is evident in the insect survivorship data; the non-significant trend was for the frogs to eat lettuce-fed lubbers more often as the experiment progressed (Fig. 2). This trend is the opposite of one that would indicate the frogs were learning an aversion to lettuce-fed lubbers. Again, these data suggest that lettuce-fed lubbers are palatable to frogs. The release of an attacked lubber by frogs, and subsequent avoidance of lubber prey, would be evidence that endogenous compounds of lubbers may deter frogs. Instead, our frogs repeatedly ate lettuce-fed lubbers (Fig. 2); some frogs ate at least ten lettuce-fed lubbers over the training and experimental periods. Frogs that ate lettuce-fed lubbers did not, from our observations, show any adverse effects from feeding on lubbers. This indicates that northern leopard frogs do not learn aversions to the endogenous chemical defenses of lettuce-fed lubbers. Thus, endoge-

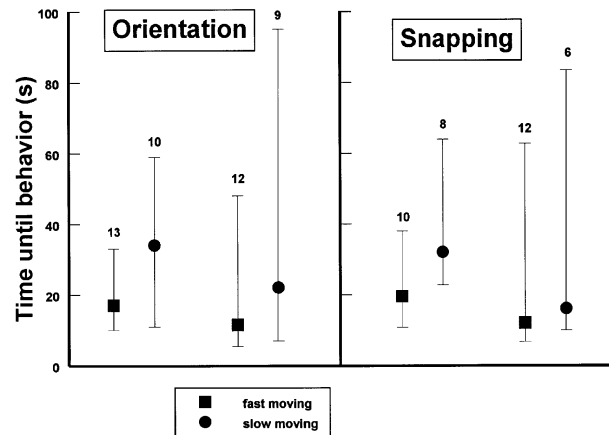


Fig. 5 Times of first orientation to and first snap at lubbers by northern leopard frogs. Each group of data is represented by the median and the interquartile range; numbers above each group of data are the number of frogs (out of 23) that exhibited the behavior. The frogs oriented towards and snapped at fast-moving lubbers significantly sooner than at slow-moving lubbers (Wilcoxon matched-pairs signed-ranks test)

nous deterrent chemicals of lubbers do not appear to be important for lubber defense from frogs.

Lubbers move more slowly than crickets

Lubbers moved about five times more slowly than crickets in both the presence of a plastic frog and in the presence of a live frog under a beaker (with a pour spout hole to allow passage of air; Table 1). Even in our experiment 1 trials, in which we offered lubbers directly to frogs (without a glass barrier between them), we observed lubbers moving much more slowly than crickets. These data suggest that the slow-movement behavior of lubbers, and perhaps chemically defended insects in general, is quantifiable and indeed different from the movement behavior of other insects. Further, this behavior appears to persist in the presence of a potential predator. In the presence of a plastic frog or a live frog under a beaker, lubber movement was the same.

We picture slow movement as a consistent movement pattern in lubbers. In other words, we believe lubbers move slowly both in the presence and absence of predators. In our experiment 2c, lubbers in the presence of a live frog under a beaker did not move significantly differently than lubbers in the absence of predators (Table 2). We hypothesize that slow-moving, chemically defended insects do not change their movement behavior in the presence of predators. However, the non-significant trend in experiment 2c was for lubbers to move more slowly in the presence of frogs. This trend was surprising. It contradicts our observation that, when collecting lubbers, they typically attempt to jump away from the collector. The possibility that lubbers may move even more slowly in the presence of small vertebrate predators warrants further study.

The evolutionary origins of slow movement in lubbers are unknown. It may have evolved in response to vertebrate predation pressures, or it may be the result of some physiological constraint that has serendipitous defensive benefits. We know of two other members of the family Acrididae that are slow moving and chemically defended (*Taeniopoda eques*, Whitman et al. 1985; *Dactyloptum variegatum*, Neal et al. 1994). Most Acrididae are highly active, but slow movement appears in three chemically defended Acrididae. This coincidence suggests that slow movement may be most beneficial in concert with chemical defense. Hence, we hypothesize that the defensive benefits of slow movement and unpalatability are additive.

Slow movement enhances lubber survivorship

Our results from experiments 1 and 2 suggest that (1) endogenous deterrent chemicals are not responsible for the defense of lettuce-fed lubbers from frogs and (2) lubbers move more slowly than crickets. Hence, we have a system in which a motion-oriented predator does not appear to be deterred by the chemical defenses of a slow-moving, aposematic insect. This gave us the opportunity to test the role of prey slow movement in encounters with predators, without the confounding influence of chemical deterrence. In experiment 3, frogs ate slow-moving lubbers significantly less than fast-moving lubbers, oriented towards slow-moving lubbers significantly later than toward fast-moving lubbers, and snapped at slow-moving lubbers significantly later than at fast-moving lubbers (Fig. 5). In addition, frog attack patterns tended to mirror lubber movement patterns. Lubbers moved most in the first 10 s of our experiment 2 trials. Frogs attacked most in the first 30 s of experiment 1 trials. This is further evidence that insect movement releases frog attacks, and lubber slow movement often fails to release these frog attacks. Thus, the slow movement of lubbers appears to significantly affect survivorship.

Chemically defended insects are often slow moving (Whitman et al. 1990), and it was previously believed that chemically defended species did not need fast movement to escape. Our data indicate an additional advantage. Slow movement may benefit chemically defended insects by failing to release predatory responses in certain motion-oriented predators. This movement behavior is distinct from an absence of movement, in which the insect "freezes," presumably to avoid visual detection by the predator. Lubbers have aposematic coloration and are highly conspicuous. In combination with slow movement, however, an aposematic insect may be detected but still survive an encounter with a motion-oriented predator, because the insect's slow movement fails to release the predator's attack response.

The evolution of the CDS

In our experiments, slow movement was advantageous without any additional chemical defense; this has implications for the evolution of the CDS. Specifically, in systems with only motion oriented predators (e.g., frogs, lizards, birds), it is possible that slow movement evolved before chemical defense. Alternatively, an insect chemically defended from one type of predator (e.g., birds) may then develop slow movement for defense from another predator (e.g., frogs). We believe it more likely that, in most CDS insects, some level of chemical defense evolved first and the subsequent evolution of slow movement added additional defense. We are presently testing the hypothesis that the benefits of chemical defense and slow movement are additive.

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