

Sluggish Movement and Repugnant Odor Are Positively Interacting Insect Defensive Traits in Encounters with Frogs

John D. Hatle,^{1,2} Brent A. Salazar,¹ and Douglas W. Whitman¹

Accepted January 10, 2001; revised March 7, 2001

Sluggish movement is common in chemically defended insects. We have recently shown that sluggish movement can be beneficial to prey when it fails to release the attack response of an ambush (=motion-oriented) predator. Here, we test the hypothesis that sluggish movement and chemical defense (i.e., repugnant odor) together are more defensive than either alone. We manipulated the movement and odor of lubber grasshoppers to produce four prey types: (1) sluggish-moving and high odor, (2) sluggish-moving and low odor, (3) fast-moving and high odor, and (4) fast-moving and low odor. We then offered these prey to frogs. In two independent experiments, frogs attacked prey type 1 (i.e., sluggish-moving and high-odor prey) significantly later than they attacked the other prey types. Hence, the defenses of sluggish movement and repugnant odor can act together to produce a prey that is better defended than prey with either defense alone. This may help explain why these two traits commonly cooccur in insects.

KEY WORDS: defensive ensemble; chemical defense; aposematic; gregarious; lubber grasshopper; ambush predator.

INTRODUCTION

In insects, chemical defense is often associated with an ensemble of traits, including aposematic coloration, gregarious behavior, diurnal activity, and

¹Behavior, Ecology, Evolution, and Systematics Section, Department of Biological Sciences, Illinois State University, Normal, Illinois 61790-4120.

²To whom correspondence should be addressed. Fax: 309-438-3722. E-mail: jhatle@ilstu.edu.

sluggish movement (Pasteels *et al.*, 1983), collectively known as the chemical defense syndrome (CDS) (Whitman *et al.*, 1985). The defensive benefits of chemical defense (e.g., Eisner, 1970; Blum, 1981; Evans and Schmidt, 1990) and aposematic coloration (e.g., Gittleman and Harvey, 1980; Roper and Wistow, 1986) are well documented. The individual protection due to aggregation has been more recently demonstrated (e.g., Sillén-Tullberg, 1990; Alatalo and Mappes, 1996; Gamberale and Tullberg, 1996, 1998). In contrast, potential defensive benefits of sluggish movement have been largely overlooked.

Sluggish movement is common in aposematic insects but is also observed in other animals, such as sloths, chameleons, poison dart frogs, and banana slugs. Sluggish movement can be quantified and is distinct from both lack of movement and rapid escape movement (Chai and Syrgley, 1990; Hatle and Faragher, 1998). For example, unpalatable butterflies fly slower than palatable butterflies (Chai and Syrgley, 1990; Marden and Chai, 1991). Because sluggish-moving insects often have conspicuous coloration and behavior, they do not appear to be attempting to prevent detection by predators. The traditional explanation for the existence of sluggish movement in aposematic insects, implicit in previous work (Chai and Syrgley, 1990; Marden and Chai, 1991), is that these insects do not need to flee potential predators because they have an effective chemical defense. We recently provided evidence for an alternative (or additional) explanation, that sluggish movement can, by itself, act as a defense. In our experiments, ambush predators attacked sluggish-moving insects significantly less and significantly later than they attacked motion-induced conspecific insects. These results suggested that conspicuous insects can have increased survival in encounters with ambush predators if they move sluggishly enough to fail to release the attack response of the predator (Hatle and Faragher, 1998).

Because sluggish movement in aposematic insects often occurs in concert with chemical defense, an interaction between these two defenses may exist. The nature of this putative interaction is important for understanding this defensive ensemble. A positive interaction between the defensive traits (i.e., greater defense with both traits than with either alone) in extant insects would suggest that the existence of one trait might stimulate the evolution of the second. Hence, we sought to test the interaction of these defenses.

In this paper, we use lubber grasshoppers and frogs as a model predator/prey system to test the hypothesis that sluggish movement and chemical defense (i.e., repugnant odor) interact positively. We paired eastern lubber grasshoppers, *Romalea microptera* (Beauvois) (= *guttata* Houttuyn; hereafter, lubbers) with northern leopard frogs, *Rana pipiens* (hereafter, frogs). Lubbers are aposematically colored (Whitman 1990), chemically defended

from ants (Jones *et al.*, 1989; Hatle and Spring, 1998) birds, and lizards (Whitman, 1990; Whitman *et al.*, 1990, 1992), and sluggish-moving (Hatle and Faragher, 1998). Frogs are motion-oriented, ambush hunters (Ewert, 1987) and will starve rather than feed in a cage filled with unmoving prey (Roth, 1986). In concert with their primary visual orientation, frogs can also use olfaction to modify their predatory responses. For example, blinded frogs were able to locate, snap at, and consume mealworms (Shinn and Dole, 1978). In addition, frogs can learn to use odor to discriminate between prey items that are visually identical (Sternthal, 1974; Rice and Taylor, 1995). In sum, lubbers can be used as models for aposematic, sluggish insects, and frogs can be used as models for predators that use both motion and odor in predatory decisions.

To test our hypothesis, we manipulated the movement and odor of lubbers to produce four types of prey: (1) sluggish-moving and high odor, (2) sluggish-moving and low odor, (3) fast-moving and high odor, and (4) fast-moving and low odor. Hereafter, we refer to these prey as “sluggish+high odor,” “sluggish+low odor,” “fast+high odor,” and “fast+low odor,” respectively.

METHODS

Experiment 1: Do the Defenses of Sluggish Movement and Acetic Acid Interact Positively?

Pilot Studies

To give lubbers a chemical defense that would have a repugnant odor, we used glacial acetic acid. One day before the experiments, we glued 6-mm-diameter filter paper disks on the dorsal abdomens of lubbers. Onto these disks, we loaded 5 μ l of concentrated acetic acid. The discs dispersed the acid, increasing the evaporative surface area and intensifying its odor. We chose acetic acid because it is volatile, with a strong, identifiable odor, and because it is a component of the defensive secretions of several arthropods, most notably vinegar weevils, ants, and true bugs (Blum, 1981; Osborn and Jaffe, 1998). We chose 5 μ l as the dosage because it was not strong enough to prevent attack but was a mild deterrent. Attacking frogs often thrashed their heads, suggesting aversion. However, during the experiment, the frogs consumed all acetic acid loaded lubbers that were attacked. Therefore, this dosage resulted in a level of chemical deterrence similar to the behavioral deterrence in our predator/prey system.

Experimental Animals

We obtained third-instar lubbers from our laboratory colony (Whitman, 1986). Grasshoppers were kept at 14:10-h L:D and $26 \pm 2^\circ\text{C}$. We fed the grasshoppers Romaine lettuce (*Lactuca sativa* var. Romaine) and oatmeal, supplemented with green onions (*Allium cepa* var. shallots) and green bean pods (*Phaseolus vulgaris*).

We purchased *R. pipiens* from Wm. A. Lemberger Co. (Oshkosh, WI) and verified their identity by dissection. The frogs were initially kept communally at $21 \pm 2^\circ\text{C}$ and were fed two to three crickets per frog twice weekly; adult crickets and third-instar lubbers are of similar size. About 10 days before the start of Experiment 1, we transferred 18 frogs [mass (mean \pm SE) = 34.9 ± 0.3 g] to individual $30 \times 30 \times 10$ -cm plastic containers with 150 ml pea gravel and 250 ml aged tap water at $22 \pm 2^\circ\text{C}$ and 14:10-h L:D. Each frog was offered three acetic acid loaded lubbers in three separate trials before the beginning of Experiment 1. Three feedings is sufficient for frogs to learn whether or not a prey item is palatable (Sternthall, 1974; Rice and Taylor, 1995; Hatle and Faragher, 1998). Therefore, all the frogs used in this study were familiar with acetic acid loaded lubbers as prey. The last feeding was always 3 days before the beginning of the experiment, to ensure that all frogs began the experiment at similar hunger levels.

Feeding Trials

We tested whether the defensive traits of sluggish movement and acetic acid odor interact in encounters with frogs by presenting frogs with four types of lubber prey: (1) sluggish+acid, (2) sluggish+water, (3) fast+acid, and (4) fast+water. To manipulate prey item movement, we tied thread around the thorax of a lubber and attached this thread above the experimental arena (Hatle and Faragher, 1998). We adjusted the thread so that the lubber could stand normally and move a few centimeters on the floor of the arena. Next to the attachment point of the thread, we placed a small electric motor. In “fast-moving” trials, the motor’s rotating lever arm plucked the thread and therefore jiggged the lubber ~ 1 cm up and back down about once every 1.2 s. This rate of movement effectively releases toad attack responses (Borchers *et al.*, 1978). The resulting movement was “jerky,” which is in direct contrast to sluggish movement, which is fluid. In “sluggish-moving” trials, the motor was placed so that the lever arm missed the thread and the lubber was not moved. Sluggish-moving trials allowed the lubber to exhibit relatively natural movements.

We conducted a trial by transferring the frog to an 11-liter glass arena equipped with the insect motion device. Each trial was a no-choice test with only one prey item available. We did not use choice tests because the point source of the odor could be unclear. The frog was placed under an opaque container until the trial began. The lubber was set up on the motion device (~10 cm from the frog) and loaded with acid or water, and the trial begun immediately by removing the opaque container from above the frog and starting the timer. We measured (1) the time the frog first turned toward or moved toward the prey (=orientation) and (2) the time the frog first projected its tongue at or lunged at the prey (=snap). In trials in which orientation was not distinct, we assigned the same time to both orientation and snapping. Importantly, orientation is not a measure of mere detection but, instead, is the first step in the sequence of frog predatory behaviors. Each trial ended when the prey's head, thorax, and abdomen were completely inside the frog's mouth or at the arbitrary time limit of 300 s, whichever occurred first. We randomly selected 18 orders of prey presentation and assigned these to the 18 frogs in the experiment.

Statistical Analyses

We analyzed predator time until first orientation with the Kaplan–Meier time-failure analysis, which is appropriate for time-dependent data (Lee, 1980; Hatle and Faragher, 1998). For each of the four prey types, we generated a response-time estimate curve (e.g., Fig. 1). This curve is a representation of the responses of the population of frogs to the particular prey type. We constructed the curves using 5-s time intervals, so that each 5-s period during a trial was a new datum. We compared these curves using chi-square pairwise tests (White and Garrolt, 1990). This comparison takes into account both the time of each response and the number of responses. The analysis tests for differences in the areas under the response curves. Throughout the paper, we refer to frogs attacking “later” for a delayed time of attack and/or a reduced number of prey attacked. Predators that do not attack a prey by the arbitrary time limit of a trial can be considered to “attack later” than predators that did attack the prey item. Because we used six pairwise comparisons within a behavior, $\alpha = 0.0083$ (Bonferroni's adjustment). We analyzed snapping times identically to orientation times. For a graphical explanation of this type of analysis, see Hatle and Faragher (1998). We analyzed insect survival data by comparing the total number of each prey type eaten. For each trial, we assigned a value of 1 if the insect survived or a value of 0 if the insect was killed. We then performed an ANOVA by prey type.

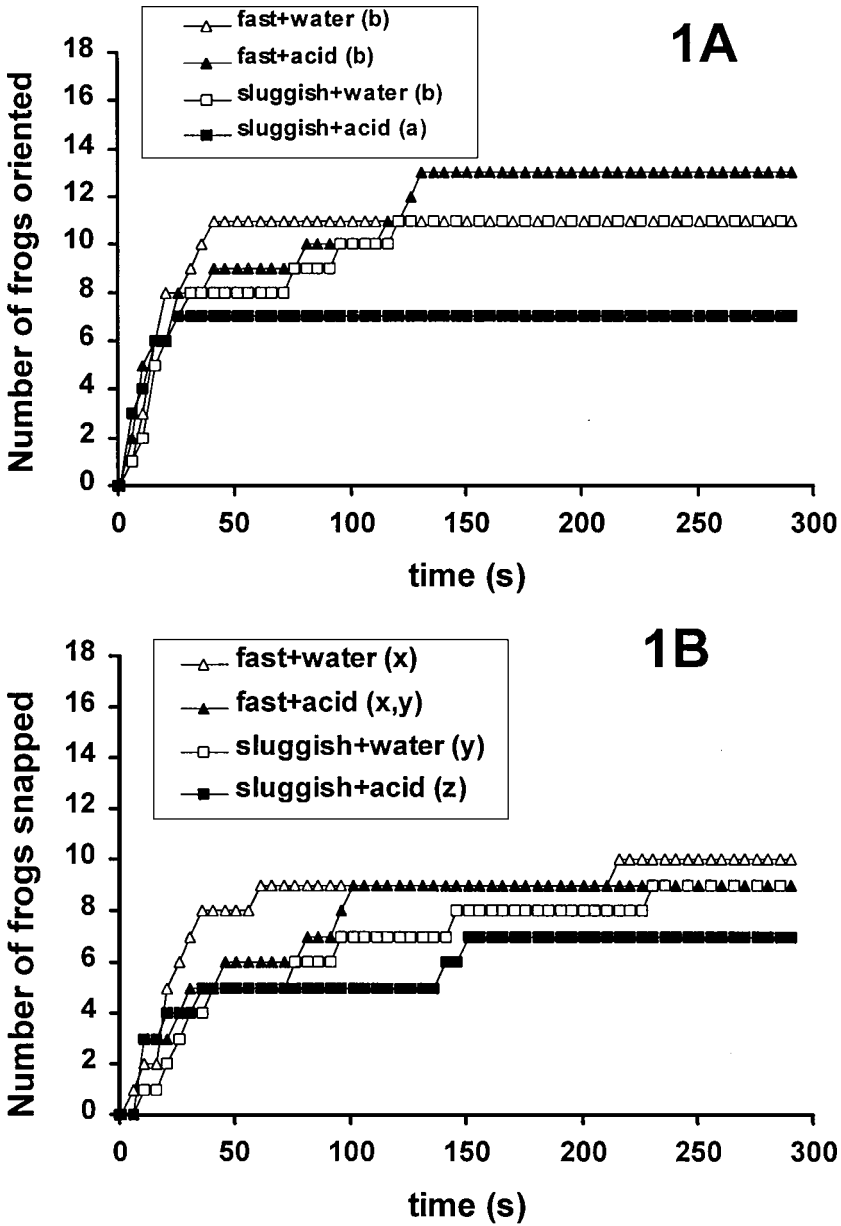


Fig. 1. (A) Response curve estimates of times until frog orientations in response to eastern lubber grasshopper prey items. Letters in the legend box indicate significant differences. Northern leopard frogs ($N = 18$) were each tested once with each prey type in a randomized experiment. (B) Response curve estimates of times until frog snapping in response to eastern lubber grasshopper prey items.

Experiment 2: Are Sluggish+Onion Prey Better Defended than Sluggish+Lettuce Prey?

We tested whether grasshopper diet could influence the defense of the insect and delay frog attacks. We collected first-instar lubbers near Lydia, Louisiana, and reared them in the laboratory at $30 \pm 2^\circ\text{C}$, RH $\sim 50\%$, and 14:10-h L:D. Lubbers were fed Romaine lettuce, green onions, green beans, apple, and Purina Cricket Chow. Starting 10 days before the beginning of Experiment 2, we fed one group of lubbers almost exclusively onion. We fed a second group of lubbers lettuce, supplemented with small amounts of green beans and apple, but never onion. We were confident that the onion-fed group contained onion compounds because we could smell the distinct onion odor when handling them but not when handling lettuce-fed lubbers. All lubbers used in Experiment 2 were third instars.

The 18 frogs used in Experiment 2 were a separate group, obtained and maintained identically to the frogs used in Experiment 1. These frogs had never encountered lubbers as prey. Three days before the beginning of Experiment 2, all frogs were fed two crickets. This experiment was counter-balanced in a 2-day mixed design. We conducted each trial in the frog's home container and collected data as in Experiment 1. The arbitrary time limit for each trial was 180 s.

Statistical Analyses

We analyzed predator time until first orientation and first snap as per Experiment 1, with $\alpha = 0.05$. Over the course of the experiment, each frog was offered two of each prey item. To construct response curves, for each frog we summed the times of orientation for both trials of a particular prey type. We analyzed insect survival data by testing total lettuce-fed lubbers eaten versus total onion-fed lubbers eaten by each frog with a one-tailed paired *t* test.

Experiment 3: Are Fast+Onion Prey Better Defended than Fast+Lettuce Prey?

We tested whether diet could influence the defense of fast-moving prey from frogs. We purchased adult house crickets (*Acheta domesticus*) from Flukers Cricket Farm (Baton Rouge, LA) and kept them under identical conditions to the lubbers in Experiment 2. We fed the crickets only Purina

Cricket Chow. The 22 frogs used in Experiment 3 were a separate group, obtained and maintained identically to the frogs used in Experiment 1.

We used two types of cricket prey items for Experiment 3. For both prey items, we removed the wings from a cricket and then glued a 6-mm-diameter paper disk to the dorsal thorax. Immediately before a trial, we saturated the filter paper disk with either homogenized Romaine lettuce or homogenized green onion. Then, we immediately began the feeding trial. Experiment 3 was designed, conducted, and analyzed identically to Experiment 2, except the arbitrary time limit for each trial was 300 s.

Experiment 4: Do the Defenses of Sluggish Movement and Onion-Feeding Interact Positively?

We collected and maintained lubbers for Experiment 4 identically to those in Experiment 2, and we tested them as third and fourth instars. Starting 11 days before the beginning of Experiment 4, we developed a lettuce-fed group and an onion-fed group as in Experiment 2.

We obtained and maintained the frogs identically to Experiment 2. Nine frogs from Experiment 2 also were used in Experiment 4. All 18 frogs used in Experiment 4 were, at the beginning of the trials, familiar with onion-fed lubbers as food. All experimental designs, feeding trials, and statistical analyses were identical to those in Experiment 1, except that the arbitrary time limit for trials was 180 s.

RESULTS

Experiment 1: Do the Defenses of Sluggish Movement and Acetic Acid Interact Positively?

Frog Orientations

We conducted Experiment 1 to determine whether predatory behaviors of frogs are deterred by the interaction of sluggish movement and a strong, artificial, chemical defense. Construction of Kaplan–Meier time–failure response curves and the appropriate pairwise comparisons revealed that the frogs oriented toward sluggish+acid lubbers significantly later than they oriented toward all three other prey types (Fig. 1A) (six pairwise tests, with all χ^2 's > 16.6, $df = 1$, and all P 's < 0.001). No other pairwise comparisons of response curves for orientations were significantly different.

Frog Snaps

Construction of response curves and the appropriate pairwise comparisons revealed that the frogs snapped at sluggish+acid lubbers significantly later than they snapped at all three other prey types (Fig. 1B) (three pairwise tests, with all χ^2 's > 9.0 , $df = 1$, and all P 's < 0.005). In addition, the frogs snapped at sluggish+water lubbers significantly later than they snapped at fast+water lubbers ($\chi^2 > 10.5$, $df = 1$, $P < 0.005$). No other pairwise comparisons of response curves for snappings were significantly different.

Insect Survivorship

There were no significant differences in insect survivorship among any of the four types of prey ($F_{3,56} = 0.414$, $P = 0.74$). The numbers of lubbers out of 18 that survived the 300-s trials were 11 sluggish+acid lubbers, 9 sluggish+water lubbers, 9 fast+acid lubbers, and 8 fast+water lubbers.

Experiment 2: Are Sluggish+Onion Prey Better Defended than Sluggish+Lettuce Prey?*Frog Responses*

We conducted Experiment 2 to develop a system in which the endogenous chemical defenses of lubbers could be naturally augmented to a sufficient degree to deter predatory behaviors of frogs. Onion-fed lubbers had a distinct odor of onion, which was lacking in lettuce-fed lubbers. In addition, all prey in this experiment were lubbers that did not have their movement manipulated; hence, all prey in this experiment were sluggish-moving. Construction of response curves and the appropriate pairwise comparisons revealed that the frogs oriented toward (Fig. 2A) ($\chi^2 = 40.6$, $df = 1$, $P < 0.001$) and snapped at (Fig. 2B) ($\chi^2 = 63.4$, $df = 1$, $P < 0.001$) onion-fed lubbers significantly later than lettuce-fed lubbers. We infer that lubber diet influenced the predatory behaviors of frogs.

Insect Survivorship

Onion-fed lubbers did not have higher survivorship than lettuce-fed lubbers ($t = 1.17$, $df = 17$, $P = 0.130$). The numbers of lubbers out of 36 that survived the 180-s trials were 20 lettuce-fed lubbers and 16 onion-fed lubbers.

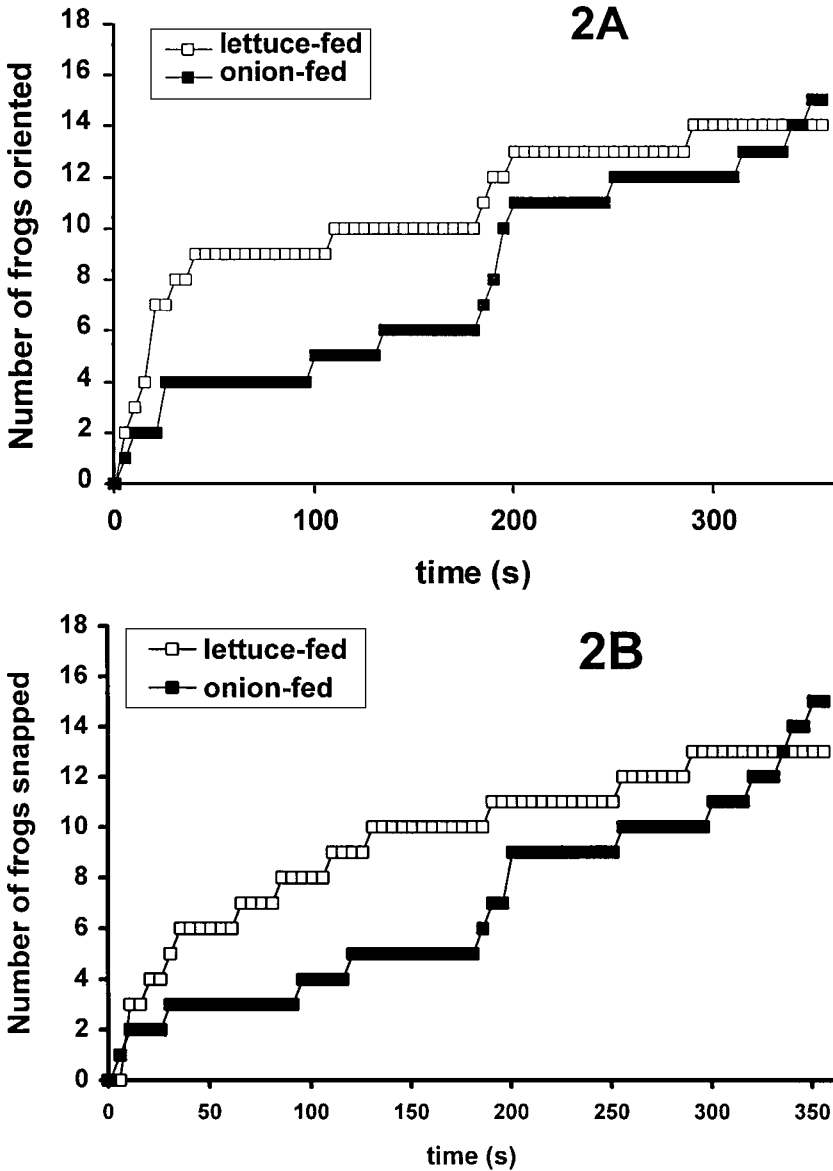


Fig. 2. (A) Response curve estimates of frog orientations in response to third-instar eastern lubber grasshopper prey items. Northern leopard frogs ($N = 18$) were each tested twice in a counter-balanced experiment. The arbitrary time limit of each trial was 180 s. Data are combined from 2 days of experiments. The frogs oriented toward onion-fed lubbers significantly later than lettuce-fed lubbers. (B) Response curve estimates of frog snappings in response to third-instar eastern lubber grasshopper prey items. The frogs snapped at onion-fed lubbers significantly later than at lettuce-fed lubbers.

Experiment 3: Are Fast+Onion Prey Better Defended than Fast+Lettuce Prey?

Frog Responses

We conducted Experiment 3 to determine whether onion odor alone (without sluggish movement) was sufficient to delay predatory behaviors of frogs. All prey in this experiment were crickets and hence were fast-moving. Construction of response curves and the appropriate pairwise comparisons revealed that the frogs oriented toward (Fig. 3A) ($\chi^2 = 2.7$, $df = 1$, $P > 0.10$) and snapped at (Fig. 3B) ($\chi^2 = 2.1$, $df = 1$, $P > 0.10$) onion-loaded crickets similarly to lettuce-loaded crickets. We infer that repugnant odor may not influence frog predatory behavior toward fast-moving prey.

Insect Survivorship

Onion-loaded crickets did not have higher survivorship than lettuce-loaded crickets ($df = 21$, $P = 0.164$) in feeding trials with frogs. The numbers of crickets out of 44 that survived the 300-s trials were 42 onion-loaded crickets and 42 lettuce-loaded crickets.

Experiment 4: Do the Defenses of Sluggish Movement and Onion-Feeding Interact Positively?

Frog Orientations

We conducted Experiment 4 to determine whether predatory behaviors of frogs are deterred by the interaction of sluggish movement and a naturally augmented chemical defense. Construction of response curves and the appropriate pairwise comparisons revealed that the frogs oriented toward sluggish+onion lubbers significantly later than they oriented toward all three other prey types (Fig. 4A) (six pairwise tests, with all χ^2 's > 22 , $df = 1$, and all P 's < 0.001). In addition, the frogs oriented toward sluggish+lettuce lubbers significantly later than they oriented toward fast+onion lubbers ($\chi^2 = 9.65$, $df = 1$, $P < 0.005$). No other pairwise comparisons of response curves for orientations were significantly different.

Frog Snaps

Construction of response curves and the appropriate pairwise comparisons revealed that the frogs snapped at sluggish+onion lubbers significantly later than they snapped at fast+onion lubbers (Fig. 4B) ($\chi^2 > 31.3$,

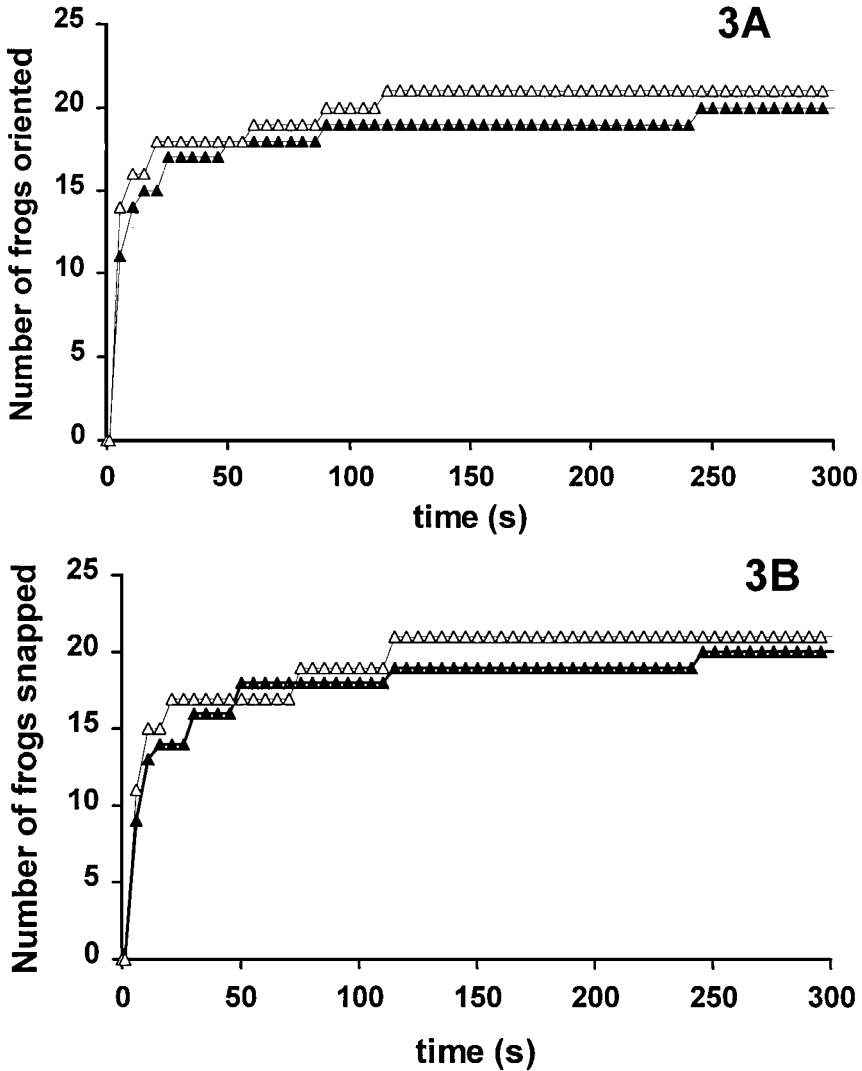


Fig. 3. (A) Response curve estimates of frog orientations in response to cricket prey items. The crickets were loaded with either homogenized lettuce (open triangles) or homogenized onion (filled triangles). Northern leopard frogs ($N = 22$) were each tested twice in a counter-balanced experiment. The arbitrary time limit of each trial was 300 s. Data are combined from 2 days of experiments. The frogs oriented toward onion-loaded crickets similarly to lettuce-loaded crickets. (B) Response curve estimates of frog snappings in response to cricket prey items. The frogs snapped at onion-loaded crickets similarly to lettuce-loaded crickets.

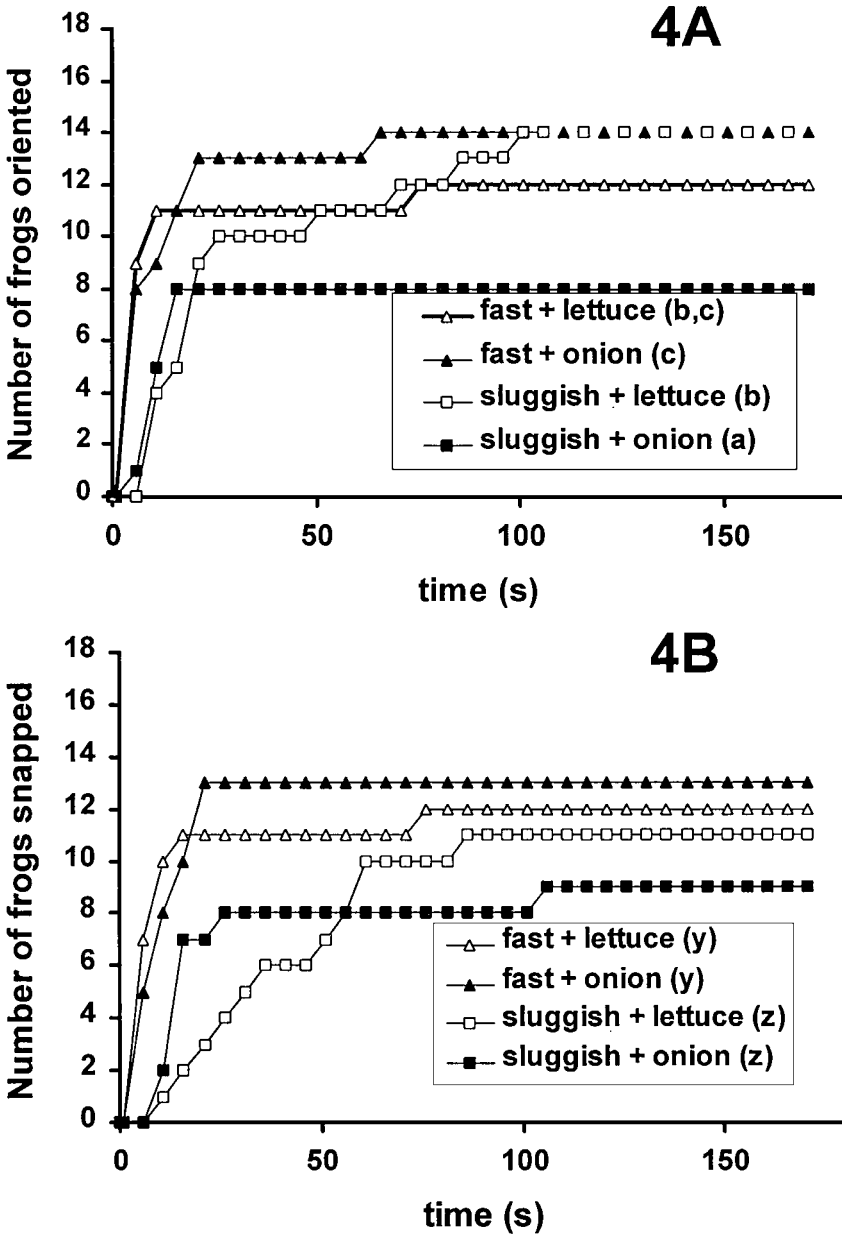


Fig. 4. (A) Response curve estimates of times until frog orientations in response to eastern lubber grasshopper prey items. Letters in the legend box indicate significant differences. Northern leopard frogs ($N = 18$) were each tested once with each prey type in a randomized experiment. (B) Response curve estimates of times until frog snapping in response to eastern lubber grasshopper prey items.

df = 1, $P < 0.001$) and fast+lettuce lubbers ($\chi^2 > 27.7$, df = 1, $P < 0.001$). In addition, the frogs snapped at sluggish+lettuce lubbers significantly later than they snapped at fast+onion lubbers ($\chi^2 = 33.8$, df = 1, $P < 0.001$) and fast+lettuce lubbers ($\chi^2 = 15.3$, df = 1, $P < 0.001$). No other pairwise comparisons of response curves for snappings were significantly different. In other words, sluggish prey were always attacked later than fast prey.

Insect Survivorship

There were no significant differences in insect survivorship among any of the four types of prey items ($F_{3,56} = 0.761$, $P = 0.520$). The numbers of lubbers out of 18 that survived the 180-s trials were 9 sluggish+onion lubbers, 7 sluggish+lettuce lubbers, 5 fast+onion lubbers, and 6 fast+lettuce lubbers.

DISCUSSION

Our results suggest that the defensive trait of sluggish movement can interact positively with chemical defense. The ultimate result of this interaction is a more highly defended prey. In our experiments, predatory behaviors of frogs were significantly delayed by sluggish+high odor prey (Figs. 1 and 4). We believe that in nature, this delay in predatory responses could increase lubber survivorship by increasing the amount of time in which they could escape. We hypothesize that the interaction of sluggish movement and repugnant odors is important in the survival of chemically defended prey in nature.

Because there are many sluggish-moving and repugnant-smelling arthropods (Blum, 1981; Whitman *et al.*, 1990), and because many arthropods (Gillot, 1995), reptiles (Cooper, 1990), amphibians (Duellman and Trueb, 1986), and mammals (Langely and Knapp, 1982; Vaughan, 1986) use both prey movement and odor in predatory decisions, the results of this paper have broad implications. Those prey that have defensive ensembles involving both sluggish movement and repugnant odor are likely to gain benefits in encounters with predators that use both movement and odor in predatory decisions.

Repugnant Odor Did Not Delay Attacks of Fast-Moving Prey by Motion-Oriented Predators

In our study, repugnant odors were unable to deter frogs from attacking fast-moving prey. The frogs attacked all fast+high odor prey similarly to fast+low odor prey (Figs. 1, 3, and 4). Importantly, the levels of chemical

defense used in Experiments 1 and 4 were sufficient to deter frogs from sluggish-moving prey (see below). Langely and Knapp (1982) found that grasshopper mice attacked crickets even when they carried an odor associated with a conditioned aversion. They concluded that the motivation of the mice to attack the moving prey overrode the learned aversion due to the odor. These results are similar to our results for frog attacks of fast+high odor prey. Together, these results imply that ambush predators may not be deterred by repugnant odors alone.

Repugnant Odors Delayed Attacks of Sluggish-Moving Prey from Motion-Oriented Predators

In contrast, repugnant odors are able to deter motion-oriented predators from sluggish-moving prey. In our study, frogs oriented toward sluggish+high odor prey significantly later than they oriented towards sluggish+low odor prey (Figs. 1A, 2A, and 4A). In Experiments 1 and 2, the frogs also snapped at sluggish+high odor prey significantly later than they snapped at sluggish+low odor prey (Figs. 1B and 2B). In Experiment 4, the nonsignificant trend was for frogs to snap at fewer sluggish+high odor prey than sluggish+low odor prey. The snaps at sluggish+high odor prey occurred sooner than the snaps at sluggish+low odor prey, hence the comparison was not significant (Fig. 4B). In sum, our data suggest that repugnant odors can deter motion-oriented predators from sluggish-moving prey.

Taken together, these results suggest that repugnant odors more effectively deter motion-oriented predators if the odor is in combination with sluggish movement. Sluggish movement alone can deter motion-oriented predators (Hatle and Faragher, 1998), but repugnant odors alone (at the level we tested) were insufficient to deter motion-oriented predators. We conclude that the defenses of sluggish movement and repugnant odor are hierarchical in our experimental system. In other words, the deterrent effect of repugnant odor was contingent upon sluggish movement.

How Do the Defenses of Sluggish Movement and Chemical Defense Interact?

The mechanism(s) by which sluggish movement and repugnant odor interacted in our experiments is unknown. We suggest one possible method. Sluggish prey, in contrast to fast prey, do not trigger an instant attack. This creates a slightly longer period in which the predator can evaluate the prey. During this time, the odor of the prey, which travels slower than the visual image of the prey, could more strongly signal the predator of the prey's chemical

defense. If the odor is sufficiently noxious, the predator will reject the prey. Fast prey, which elicit a more immediate reflex response from predators, do not produce this prolonged predatory decision. As a result, fast prey would gain less benefit from repugnant odors.

The chemical defense syndrome (CDS) (Whitman *et al.*, 1985), including defensive chemicals, aposematic coloration, aggregation, and sluggish movement, is common in insects (Pasteels *et al.*, 1983). The following pairs of CDS defenses have been shown to interact positively: defensive chemicals and aposematic coloration (e.g., Pearson, 1985; Roper and Redston, 1987), defensive chemicals and aggregation (Alatalo and Mappes, 1996), defensive chemicals and sluggish movement (this paper), and aposematic coloration and aggregation (Gamberale and Tullberg, 1996, 1998; Hatle and Salazar, 2001; Sillén-Tullberg, 1990). Hence, CDS traits appear to interact positively with other CDS characters. If each characteristic of the syndrome works in concert with the other defensive traits, this would help explain the preponderance of this defense ensemble.

ACKNOWLEDGMENTS

We thank Susan Mopper and Daniel Povinelli for advice on experimental design and Robert Jaeger, Clinton Jeske, Susan Mopper, Jeffrey Spring, and the ISU Advanced Entomology class for critically reading the paper. J.D.H. was supported by a University of Louisiana Lafayette Doctoral Fellowship and National Science Foundation dissertation improvement grant IBN-9701082 to Jeffrey Spring and J.D.H. D.W.W. was supported by National Science Foundation Grant BIR-9510979 A000 and Department of Education Grant P094B50041.

REFERENCES

- Alatalo, R. V., and Mappes, J. (1996). Tracking the evolution of warning signals. *Nature* **382**: 708–710.
- Blum, M. S. (1981). *Chemical Defense of Arthropods*, Academic Press, New York.
- Borchers, H.-W., Burghagen, H., and Ewert, J.-P. (1978). Key stimuli of prey for toads (*Bufo bufo* L): Configuration and movement patterns. *J. Comp. Physiol.* **128**: 189–192.
- Brower, J. V. Z., and Brower, L. P. (1962). Experimental studies of mimicry. 6. The reactions of toads (*Bufo terrestris*) to honeybees (*Apis mellifera*) and their dronefly mimics (*Eristalis vinetorum*). *Am. Nat.* **96**: 297–307.
- Brower, L. P., Brower, J. V. Z., and Westcott, P. W. (1960). Experimental studies of mimicry 5. The reactions of toads (*Bufo terrestris*) to bumblebees (*Bombus americanorum*) and their robberfly mimics (*Mallphora bomboides*), with a discussion of aggressive mimicry. *Am. Nat.* **94**: 343–355.
- Chai, P., and Srygley, R. B. (1990). Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. *Am. Nat.* **135**: 748–765.

- Cooper, W. E., Jr. (1990). Prey odor detection by teiid and lacertid lizards and the relationship of prey odor detection to foraging mode in lizard families. *Copeia* **1990**: 237–242.
- Coppinger, R. P. (1970). The effect of experience and novelty on avian feeding behavior with reference to the evolution of warning coloration in butterflies. II. Reactions of naive birds to novel insects. *Am. Nat.* **104**: 323–335.
- Duellman, W. E., and Trueb, L. (1986). *Biology of Amphibians*, McGraw–Hill, New York.
- Edmunds, M. (1974). *Defence in Animals: A Survey of Anti-predator Defenses*, Longman Group, New York.
- Eisner, T. (1970). Chemical defenses against predation in arthropods. In Sondeimer, E., and Simeone, J. B. (eds.), *Chemical Ecology*, Academic Press, New York, pp. 157–218.
- Endler, J. A. (1986). Defense against predators. In Feder, M. E., and Lauder, G. V. (eds.), *Predator-Prey Relationships: Perspectives from the Study of Lower Vertebrates*, University of Chicago Press, Chicago, pp. 109–134.
- Evans, D. L., and Schmidt, J. O. (1990). *Insect Defenses*, State University of New York Press, Albany.
- Ewert, J.-P. (1987). Neuroethology of releasing mechanisms: Prey catching in toads. *Behav. Brain Sci.* **10**: 337–445.
- Gagliardo, A., and Guilford, T. (1993). Why do warningly-coloured prey live gregariously? *Proc. R. Soc. Lond. Ser. B* **251**: 69–74.
- Gamberale, G., and Tullberg, B. S. (1996). Evidence for a more effective signal in aggregated aposematic prey. *Anim. Behav.* **52**: 597–601.
- Gamberale, G., and Tullberg, B. S. (1998). Aposematism and gregariousness: The combined effect of group size and coloration on signal repellence. *Proc. R. Soc. Lond. Ser. B* **265**: 889–894.
- Gillot, C. (1995). *Entomology, 2nd ed.*, Plenum Press, New York.
- Gittleman, J. L., and Harvey, P. H. (1980). Why are distasteful prey not cryptic? *Nature* **286**: 149–150.
- Greenwood, J. J. D., Cotton, P. A., and Wilson, D. M. (1989). Frequency-dependent selection on aposematic prey: Some experiments. *Biol. J. Linn. Soc.* **36**: 213–226.
- Guilford, T. (1990). The evolution of aposematism. In Evans, D. L., and Schmidt, J. O. (eds.), *Insect Defenses*, State University of New York Press, Albany, pp. 289–351.
- Hatle, J. D., and Faragher, S. G. (1998). Slow movement increases the survivorship of a chemically defended grasshopper in predatory encounters. *Oecologia* **115**: 260–267.
- Hatle, J. D., and Salazar, B. A. (2001). Aposematic coloration of gregarious insects can delay predation by an ambush predator. *Environ Entomol.* **30**: 51–54.
- Hatle, J. D., and Spring, J. H. (1998). Inter-individual variation in sequestration (as measured by energy dispersive spectroscopy) predicts efficacy of defensive secretion in lubber grasshoppers. *Chemoecology* **8**: 85–90.
- Jones, C. G., Whitman, D. W., Compton, S. J., Silk, P. J., and Blum, M. S. (1989). Reduction in diet breadth results in sequestration of plant chemicals and increases efficacy of chemical defense in a generalist grasshopper. *J. Chem. Ecol.* **15**: 1811–1822.
- Langely, W. L., and Knapp, K. (1982). Importance of olfaction to suppression of attack response through conditioned taste aversion in the grasshopper mouse. *Behav. Neural Biol.* **35**: 368–378.
- Lee, E. T. (1980). *Statistical Methods for Survival Data Analysis*, Lifetime Learning, Belmont, CA.
- Mappes, J., and Alatalo, R. V. (1997). Effects of novelty and gregariousness in survival of aposematic prey. *Behav. Ecol.* **8**: 174–177.
- Marden, J. H., and Chai, P. (1991). Aerial predation and butterfly design: How palatability, mimicry, and the need for evasive flight constrain mass allocation. *Am. Nat.* **138**: 15–36.
- Marples, N. M., van Veelen, W., and Brakefield, P. M. (1994). The relative importance of colour, taste and smell in the protection of an aposematic insect *Coccinella septempunctata*. *Anim. Behav.* **48**: 967–974.
- Osborn, F., and Jaffe, K. (1998). Chemical ecology of the defense of two nymphalid butterfly larvae against ants. *J. Chem. Ecol.* **24**: 1173–1180.

- Pasteels, J. M., Gregoire, J., and Rowell-Rahier, M. (1983). The chemical ecology of defense in arthropods. *Annu. Rev. Entomol.* **28**: 263–289.
- Pearson, D. L. (1985). The function of multiple anti-predator mechanisms in adult tiger beetles (Coleoptera: Cinindeliidae). *Ecol. Entomol.* **10**: 65–72.
- Pearson, D. L. (1989). What is the adaptive significance of multicomponent defensive repertoires? *Oikos* **54**: 251–253.
- Rice, T. M., and Taylor, D. H. (1995). A simple test of prey discrimination that demonstrates learning in postlarval ranid frogs. *J. Herpetol.* **29**: 320–332.
- Roper, T. J., and Redston, S. (1987). Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance learning. *Anim. Behav.* **35**: 739–747.
- Roper, T. J., and Wistow, R. (1986). Aposematic coloration and avoidance learning in chicks. *Q. J. Exp. Psychol.* **38B**: 141–149.
- Shinn, E. A., and Dole, J. W. (1978). Evidence for a role for olfactory cues in the feeding response of leopard frogs, *Rana pipiens*. *Herpetologica* **34**: 167–172.
- Sillén-Tullberg, B. (1985). Higher survival of an aposematic than a cryptic form of a distasteful bug. *Oecologia* **67**: 411–415.
- Sillén-Tullberg, B. (1990). Do predators avoid groups of aposematic prey? An experimental test. *Anim. Behav.* **40**: 856–860.
- Sillén-Tullberg, B., and Bryant, E. H. (1983). The evolution of aposematic coloration in distasteful prey: An individual selection model. *Evolution* **37**: 993–1000.
- Sternthal, D. E. (1974). Olfactory and visual cues in the feeding behavior of the leopard frog (*Rana pipiens*). *Z. Tierpsychol.* **34**: 239–246.
- Vaughan, T. A. (1986). *Mammalogy*, Saunders College, Philadelphia.
- Vulinec, K. (1990). Collective security: Aggregation by insects as a defense. In Evans, D. L., and Schmidt, J. O. (eds.), *Insect Defenses*, State University of New York Press, Albany, pp. 289–351.
- Wiklund, C., and Järvi, T. (1982). Survival of distasteful insects after being attacked by naive birds: A reappraisal of the theory of aposematic coloration evolving through individual selection. *Evolution* **36**: 998–1002.
- White, G. C., and Garrold, R. A. (1990). *Analysis of Wildlife Radio-Tracking Data*, Academic Press, New York.
- Whitman, D. W. (1986). Laboratory biology of *Taeniopoda eques* (Orthoptera: Acrididae). *J. Entomol. Sci.* **21**: 87–93.
- Whitman, D. W. (1990). Grasshopper chemical communication. In Chapman, R. F., and Joern, A. (eds.), *Biology of Grasshoppers*, John Wiley & Sons, New York, pp. 357–391.
- Whitman, D. W., Blum, M. S., and Jones, C. G. (1985). Chemical defense in *Taeniopoda eques* (Orthoptera: Acrididae): Role of the metathoracic secretion. *Ann. Entomol. Soc. Am.* **78**: 451–455.
- Whitman, D. W., Blum, M. S., and Alsop, D. W. (1990). Allomones: chemicals for defense. In Evans, D. L., and Schmidt, J. O. (eds.), *Insect Defenses*, State University of New York Press, Albany, pp. 289–351.
- Whitman, D. W., Jones, C. G., and Blum, M. S. (1992). Defensive secretion production in lubber grasshoppers (Orthoptera: Romaleidae): Influence of age, sex, diet, and discharge frequency. *Ann. Entomol. Soc. Am.* **85**: 96–102.