



Research article

Survival advantage of sluggish individuals in aggregations of aposematic prey, during encounters with ambush predators

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Abstract. Movement is an important element of prey defense ensembles. The adaptive advantages of either remaining motionless or rapid escape are clear. In contrast, putative benefits are unclear for sluggish movement of aposematic prey that are neither fleeing nor avoiding detection of predators. Nonetheless, sluggish movement is common in aposematic insects. Our central hypothesis is that sluggish movement evolved in part by motion-oriented predators culling the fastest-moving insects from an aggregation. This would be particularly likely in chemically defended prey, which would deter continued predation. We test predictions of our hypothesis with feeding experiments using the sluggish, gregarious Eastern lubber grasshopper and a motion-oriented predator, the Northern leopard frog. Sluggish-moving (i.e., control) grasshoppers were significantly less likely to be eaten than fast-moving (i.e., motion-induced) grasshoppers ($p = 0.0098$). Next, non-moving grasshoppers were used as extreme sluggish-moving prey. Non-moving prey were significantly less likely to be eaten than sluggish-moving grasshoppers ($p = 0.05$). In addition, and most importantly, sluggish-moving grasshoppers in an aggregation were significantly less likely to be attacked than fast-moving grasshoppers in the aggregation ($p = 0.0156$). Finally, the survivorship of sluggish-moving grasshoppers in pairings vs. aggregations was not significantly different ($p = 0.33$). Our results demonstrate that the fastest-moving individual in an aggregation of aposematic insects is more likely than sluggish cohorts to be attacked by motion-oriented predators. This survival disadvantage for fast-moving, gregarious prey could create a selection pressure for the evolution of sluggish movement as a defense mechanism in aposematic, gregarious prey.

Key words: anti-predator behaviors, chemical defense, frogs, gregarious, lubber grasshopper, movement

Introduction

Movement is an important element of prey defense ensembles, but has been largely overlooked (Srygley, 1999). In fact, perhaps the two most common insect defense strategies involve motion: remaining motionless (and undetected) and fleeing (Edmunds, 1974). Avoiding detection and running away are

common and effective defense strategies in insects, and their adaptive advantages are clear. Nonetheless, many aposematic prey are neither motionless nor quick, but exhibit sluggish movement; these prey appear neither to be avoiding detection by nor running away from predators. Putative benefits are unclear for sluggish movement in aposematic prey vis-à-vis predators. Hence, the function and evolution of sluggish movement are of interest.

Sluggish movement is exhibited by many animals, such as slugs, chameleons, efts, and toads, and it is particularly common in aposematic, gregarious insects (Pasteels *et al.*, 1983; Whitman *et al.*, 1985; Chai and Srygley, 1990; Hatle and Faragher, 1998; Hatle and Whitman, 2001). It is fluid, deliberate, and lethargic. The traditional explanation for the existence of sluggish movement in aposematic prey is that, because these prey are unpalatable, they do not need to flee predators (implied in Chai and Srygley, 1990; Srygley and Chai, 1990a, b; Marden and Chai, 1991). We have recently provided an additional explanation, which we develop further in this paper. We argue that conspicuous prey gain a survival advantage if they move slow enough to fail to release the attack response of motion-oriented (e.g., ambush) predators. The optimal release of the attack response from motion-oriented predators such as frogs (Ewert, 1987), snakes (Mattison, 1999), wolf spiders (Rovner, 1993), and praying mantids (Rilling *et al.*, 1959) requires a specific rate of prey movement (about 1 Hz for toads; Borchers *et al.*, 1978). Sluggish-moving prey may simply move slower than this rate, and thus may not trigger an attack response in such predators (Hatle and Faragher, 1998). In this way, sluggish movement could impart defensive benefits.

Although we have previously demonstrated that sluggish movement can be beneficial in encounters with motion-oriented predators, the evolution of sluggish movement is still unclear. In this paper, we present one possible scenario for the evolution of sluggish movement as a defense in aposematic, gregarious prey. Our central hypothesis is that sluggish movement evolved in part from predation pressure exerted by motion-oriented predators culling the fastest-moving insects from aggregations. This would be particularly likely in chemically defended prey, because predators would be deterred from continued foraging after being poisoned. Predation of the fastest individuals in a group would create a selection pressure to move slowly relative to gregarious cohorts.

We tested our hypothesis using a sluggish, gregarious, aposematic, chemically defended insect (Whitman, 1990; Hatle and Faragher, 1998), the Eastern lubber grasshopper (*Romalea microptera* [= *guttata*]). Lubber grasshoppers have effective chemical defenses against birds (Yosef and Whitman, 1992), ants (Jones *et al.*, 1989; Hatle and Spring, 1998), and lizards (Whitman, 1990; but see Hatle and Townsend, 1996). In contrast, some frogs are not deterred by innate defenses of lubber grasshoppers (Hatle and Faragher, 1998). Juvenile lubbers are highly gregarious and can be found in aggregations of about 50

grasshoppers/100 cm². Older instars and adults are not as gregarious, but tend to clump. Importantly, all stages of lubber grasshoppers are sluggish-moving (Whitman, 1988a, 1990).

Here, we test three predictions of our central hypothesis using third instar lubber grasshoppers and a model motion-oriented predator, the Northern leopard frog (*Rana pipiens*).

Prediction 1: Third instar lubber grasshoppers will vary in repeatable rate of movement (because selection requires heritable variation).

Prediction 2: During encounters with motion-oriented predators, survivorship of lubbers will be inversely related to rate of movement. Specifically, sluggish-moving grasshoppers will have significantly higher survivorship than faster-moving grasshoppers. In contrast, sluggish-moving grasshoppers will have significantly lower survivorship than non-moving grasshoppers.

Prediction 3: During encounters with motion-oriented predators, sluggish-moving grasshoppers in an aggregation will have significantly higher survivorship than fast-moving grasshoppers in the same aggregation.

In addition, we conducted one follow-up test that is related to our central hypothesis but that is not a requirement of the hypothesis. We tested whether the defensive benefits of sluggish movement increase with group size. We predicted that sluggish-moving grasshoppers in a group of seven will have significantly higher survivorship than sluggish-moving grasshoppers in a group of two.

Methods

Experimental animals

We reared lubber grasshoppers by established methods (Whitman, 1986; Chladny and Whitman, 1997). Grasshoppers were fed Romaine lettuce and oatmeal daily, and green beans and green onions occasionally. We used third and fourth instar lubbers. For experiments 1, 2, and 3 we used grasshoppers from our laboratory colony. In experiments 4 and 5 we used grasshoppers collected as first and second instars near Lydia, LA, USA. Because laboratory colony animals can have different behaviors than field-collected animals, we never used both laboratory-reared and field-collected grasshoppers in the same experiment.

We obtained Northern leopard frogs (hereafter, frogs) from Wm. A. Lemberger Co. (Oshkosh, WI, USA). We verified that these frogs were *R. pipiens* by dissecting representative frogs (used in experiments 2 and 5) and examining the internal genitalia. We kept frogs in individual plastic containers with 150 ml

pea gravel and 250 ml aged tap water. Twice weekly, we fed each frog three crickets. For experiments 2, 3, and 5, all the frogs were trained to feed on lubber grasshoppers through at least two feedings, which is sufficient to familiarize frogs with novel prey (Sternthal, 1974).

Experiment 1 – variability in movement rate of third instar grasshoppers

In experiment 1, we sought to quantify repeatable variation in movement rate of third instar grasshoppers (prediction 1). We tested 20 individuals, and each of these 20 was tested in five trials. For each trial, we randomly selected two grasshoppers to use as test subjects. Grasshoppers used as test subjects had molted >24 h before the experiment and were not expected to molt until >24 h after the experiment. We marked these two test subjects and then selected five additional grasshoppers that served as the aggregation. The aggregation was placed in the center of a 40 × 40 × 20 cm plexiglass box with a grid composed of 5-cm squares. The first test subject was placed 5 cm to the right of the aggregation, and the second test subject was placed 5 cm to the left of the aggregation. Next, we placed a wire screen over the entire experimental arena to prevent escapes. Immediately, we began the 300 sec trial. During each trial, for both test subjects, we counted the number of times the grasshopper's right front tarsus crossed a gridline. We took this number as a measure of the insect's rate of movement during the trial (Hatle and Faragher, 1998). Because we were most interested in the repeatability of movement rate for an individual (i.e., the random effect), we analyzed experiment 1 by considering each individual as a treatment group with five data points. We tested for differences in movement rate among individuals using a one-way ANOVA, and we report the intraclass correlation (i.e., the percentage of variance due to variation among individuals).

Experiment 2 – defense of sluggish-moving vs. fast-moving grasshoppers from frogs

In experiment 2, we tested whether sluggish-moving prey had higher survivorship than fast-moving prey, in encounters with frogs (prediction 2). Prey that move faster than extant individuals can be used experimentally to test whether sluggish movement is beneficial in comparison to fast movement, which we assume is ancestral.

In each trial, a frog ($n = 23$) was presented with both a sluggish-moving grasshopper and a fast-moving grasshopper. Each frog received two trials, and the side on which the sluggish-moving grasshopper was presented was counter-balanced.

Lubber grasshoppers are slow-moving insects. To induce lubbers with motion and create fast-moving prey, we used the method of Hatle and Faragher (1998). A grasshopper was tied around the pronotum with a segment of black thread. The thread was attached above the experimental arena so that the grasshopper could just touch the floor and move a few cm (Fig. 1). Adjacent to the attachment point of this tether, we placed an electric motor that rotated a lever arm. The motor was positioned so that the lever arm would pluck the thread and jig the grasshopper about 60 times/min. This device lifted the lubber up ~ 1 cm and then released it. We defined these prey items as fast-moving grasshoppers. The resulting movement of the grasshopper was jerky, which is in direct contrast to sluggish movement, which is fluid. Sluggish-moving grasshoppers were presented identically, except that the electric motor was moved to the side so that the lever could not pluck the tether.

To begin each trial, we transferred the frog from its home cage to the experimental arena and covered it with an opaque plastic cover. We then prepared the prey items. When both prey items were ready (Fig. 1), we uncovered the frog and began the trial. The trial ended at the arbitrary time limit of 300 sec or when the frog had a grasshopper's head, thorax, and abdomen completely in its mouth (=eaten). We measured grasshopper survivorship by counting the number of grasshoppers that were eaten. The frogs could not easily swallow tethered prey, and this occasionally caused the frog to spit out the grasshopper. Aposematic prey can survive predatory attacks (Järvi *et al.*, 1981; Wiklund and Järvi, 1982), so unless the encounter is followed to completion, conclusions about prey survivorship are tenuous. However, in previous

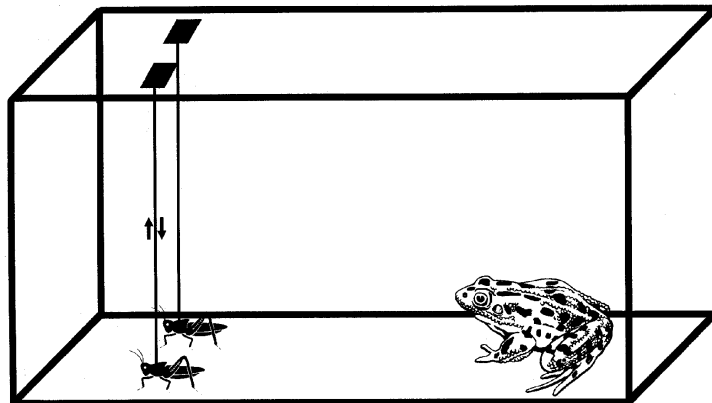


Figure 1. Sketch of our experimental setup. In a standard trial, we tied two grasshoppers and tethered them from above. We induced one with motion by plucking its tether at 1 Hz with a motor-driven lever. The other grasshopper was allowed to exhibit its natural, sluggish-moving behavior. Then, a frog was released and allowed to attack one prey item.

studies using untethered lubber grasshoppers, frogs spat out grasshoppers in <4% of trials, and all the frogs that spat out a grasshopper later consumed another grasshopper (Hatle and Faragher, 1998). Therefore, we are confident our measure of grasshopper survivorship is accurate. We compared the total number of sluggish-moving vs. fast-moving grasshoppers that survived the trials. The data were related (by frog) and not normally distributed (Kolmogorov–Smirnov normality test; $KS_{\text{fast}} = 0.315$; $KS_{\text{sluggish}} = 0.459$; both $p < 0.05$); hence, we compared sluggish-vs. fast-moving using a Wilcoxon matched-pairs test (Jaeger *et al.*, 1995).

Experiment 3 – defense of sluggish-moving vs. non-moving grasshoppers from frogs

In experiment 3, we tested whether slower-moving prey had higher survivorship than sluggish-moving prey, in encounters with frogs (prediction 2). If there exists a selection pressure for sluggish movement in aposematic, gregarious prey during encounters with motion-oriented predators, then even slower movement should be more beneficial. Prey that move slower than extant individuals can be used to test whether movement rate might still be under selective pressure. We have been unsuccessful at manipulating grasshoppers so they are more sluggish than extant grasshoppers yet still moving. Chilled grasshoppers, for example, rapidly transition from non-moving to normal movement rates. Therefore, we used dead grasshoppers as non-moving grasshoppers. Non-moving grasshoppers were frozen at $-20\text{ }^{\circ}\text{C}$ for at least 15 min, thawed, and then tied and tethered identically to the sluggish grasshopper.

In each trial, a frog ($n = 11$) was presented with two tethered prey: one sluggish-moving grasshopper and one non-moving grasshopper. Each frog received two trials, and the side of presentation for the sluggish-moving grasshopper was counter-balanced. The frogs for experiment 3 had already been used for experiment 5. Hence, they had already experienced lubber grasshoppers as prey. We statistically compared the total number of sluggish-moving vs. non-moving grasshoppers that survived the trial as in experiment 2.

Experiment 4 – defense from frogs of sluggish-moving vs. fast-moving grasshoppers in aggregations

We designed experiment 4 to test whether sluggish movement was advantageous for individual insects in an aggregation. We used 11 frogs that were naïve to lubbers as prey at the beginning of the study. During the course of experiment 4, each frog received six trials; all trials were identical except that the position of the sluggish-moving grasshopper was alternated every trial.

Our experimental setup used elements of both experiment 1 and experiment 2. The arena was identical to experiment 1, with the exception that the screen cover was not placed over the arena. The frog was placed in the arena under an opaque container, and then we prepared one sluggish-moving prey item and one fast-moving prey item (both tethered, as shown in Fig. 1). Next, five untethered, ‘gregarious’ grasshoppers were placed between the two test grasshoppers; the gregarious hoppers were positioned as per experiment 1. Immediately after the gregarious grasshoppers were ready, we removed the opaque container and started the trial. We collected data as in experiment 2, with the addition of the possibility that the frogs could eat grasshoppers in the aggregation. Trials were terminated after the frog ate a grasshopper, or at the arbitrary time limit of 300 sec. Each trial was a choice-test among one tethered sluggish-moving grasshopper, one tethered fast-moving grasshopper, and five gregarious untethered grasshoppers. This allowed a direct comparison of the two tethered grasshoppers in the trial. Statistical tests were identical to experiment 2.

Experiment 5 – frog attacks of sluggish-moving vs. fast-moving grasshoppers in pairings and in aggregations

Experiment 5 was conducted to determine whether the advantage of moving sluggishly in an aggregation is greater than the advantage of moving sluggishly in a pairing. Each frog ($n = 15$) received two prey presentations, in a random order: (1) sluggish-moving vs. fast-moving as a pairing; and (2) sluggish-moving vs. fast-moving in an aggregation. Both prey presentations were offered to each frog twice, and the side of presentation was counterbalanced. In experiment 5, some data sets were normally distributed and some were not. We chose to analyze the data with paired t -tests with the Bonferroni correction for multiple tests ($\alpha = 0.05/4 = 0.0125$). We chose the parametric test because the paired t -test and the Wilcoxon test returned the same qualitative results in all cases except one, and the data set that differed was normally distributed (Kolmogorov–Smirnov normality test; $KS = 0.35$; $p > 0.05$). This allowed us to maintain consistency within experiment 5. Fortunately, t -tests are robust to deviations from normality (Sokal and Rohlf, 1995).

In addition to measuring grasshopper survivorship in experiment 5, we also measured frog predatory behaviors. We recorded the time of first orientation and the time of first snap (= lunge) of the frog at each prey item. Orientation, snapping, and eating are sequential frog predatory behaviors (Ewert, 1987). Both the orientation data ($KS_{\text{paired}} = 0.166$; $KS_{\text{aggregated}} = 0.135$; both $p > 0.10$) and the snapping data ($KS_{\text{paired}} = 0.212$; $KS_{\text{aggregated}} = 0.201$; both $p > 0.10$) were normally distributed. Therefore, we used Student’s t -tests to analyze times to orient and snap for frogs that responded.

Results

Experiment 1 – variability in movement rate of grasshoppers

There was highly significant variation in repeatable movement rate among extant lubber grasshoppers. (Fig. 2; ANOVA; $F_{19,99} = 2.82$; $p = 0.0007$). The intraclass correlation (i.e., the percentage of variance due to variation among individuals as opposed to within individuals) was $8.33/(8.33 + 22.9) = 26.7\%$. The rate of movement of sluggish grasshoppers, which crossed about 3 grid-lines/min, appears to be much less than the rate of movement of our manipulated, fast grasshoppers, which were jiggled about 60 times/min.

Experiment 2 – defense of sluggish-moving vs. fast-moving grasshoppers from frogs

Sluggish-moving grasshoppers were significantly less likely to be eaten than fast-moving grasshoppers (Fig. 3; two-tailed Wilcoxon test; $T^+ = 61$; $p = 0.0098$). Frogs failed to eat in 23 of 46 trials.

Experiment 3 – defense of sluggish-moving vs. non-moving grasshoppers from frogs

Non-moving grasshoppers were significantly less likely to be eaten than sluggish-moving grasshoppers (Fig. 3; two-tailed Wilcoxon test; $T^+ = 21$; $p = 0.05$). Frogs failed to eat in 13 of 22 trials.

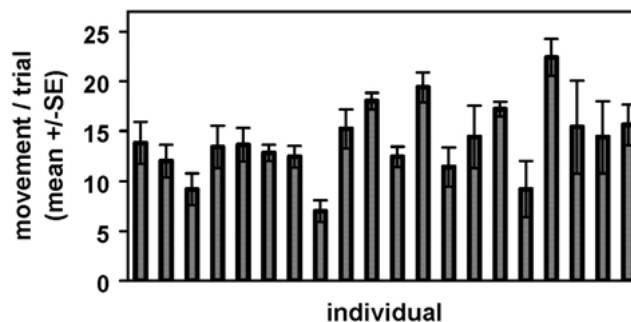


Figure 2. Rate of movement (mean \pm SE) of 20 third instar lubber grasshoppers, each tested in five 300-sec trials. We grouped seven grasshoppers together to simulate an aggregation and then measured the movement rate of two of these seven. We quantified movement rate by counting the number of times a grasshopper's right front tarsus crossed a line on a grid of 5-cm squares.

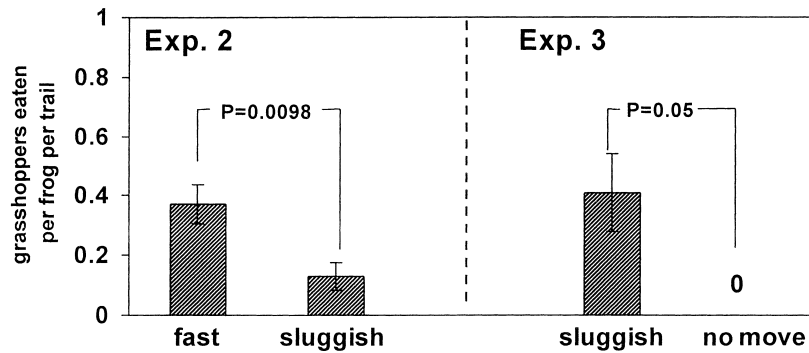


Figure 3. Survivorship of grasshoppers (mean \pm SE) with different movement rates in encounters with frogs, as per Figure 1. Sluggish-moving grasshoppers had significantly higher survivorship than fast-moving grasshoppers. Non-moving grasshoppers had significantly higher survivorship than sluggish-moving grasshoppers.

Experiment 4 – defense from frogs of sluggish-moving vs. fast-moving grasshoppers in aggregations

Sluggish-moving grasshoppers in aggregations were significantly less likely to be attacked than fast-moving grasshoppers in the aggregation (Fig. 4; two-tailed Wilcoxon test; $T^+ = 28$; $p = 0.0156$). Frogs failed to eat in 33 of 66 trials.

Experiment 5 – frog attacks of sluggish-moving vs. fast-moving grasshoppers in pairings and in aggregations

When frogs were offered prey in pairings, sluggish-moving grasshoppers were significantly less likely to be eaten than fast-moving grasshoppers (Fig. 5; two-tailed paired t -test; $t_{14} = 3.67$; $p = 0.0025$; Bonferroni corrected $\alpha = 0.0125$). When offered in an aggregation, sluggish-moving grasshoppers again were significantly less likely to be eaten than fast-moving grasshoppers (two-tailed paired t -test; $t_{14} = 3.50$; $p = 0.0035$). In comparing attacks of prey across presentations, the survivorship of fast-moving grasshoppers in pairings was not significantly different from the survivorship of fast-moving grasshoppers in aggregations (two-tailed paired t -test; $t_{14} = 2.17$; $p = 0.048$; Bonferroni corrected $\alpha = 0.0125$). The survivorship of sluggish-moving grasshoppers in pairings vs. aggregations was not significantly different (two-tailed paired t -test; $t_{14} = 1.0$; $p = 0.33$). Finally, the frogs oriented towards (two-tailed Student's t -test; $t_{21} = 5.20$; $p < 0.0001$) and snapped at ($t_{19} = 6.30$; $p < 0.0001$) fast-moving grasshoppers in a paired presentation significantly sooner than fast-moving grasshoppers in an aggregation (Table 1).

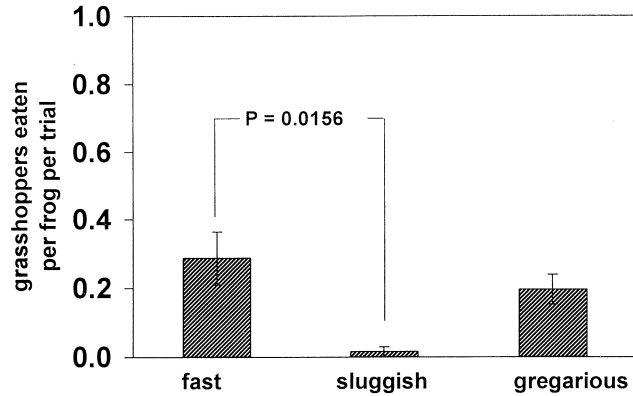


Figure 4. Survivorship (mean \pm SE) of sluggish- vs. fast-moving grasshoppers in an aggregation, during encounters with frogs. Each trial contained one sluggish-moving grasshopper and one fast-moving grasshopper, as per Figure 1. In addition, we introduced five untethered, sluggish-moving grasshoppers (the 'gregarious' grasshoppers) between the two tethered grasshoppers before we began the trial. Hence, there were five untethered gregarious grasshoppers, one tethered sluggish grasshopper, and one tethered fast grasshopper per trial. The graph shows attacks of any of the five gregarious grasshoppers during a trial; in other words, there were fivefold more gregarious grasshoppers to attack than either fast or sluggish grasshoppers. Frogs were allowed to attack only one insect per trial. Sluggish grasshoppers had significantly higher survivorship than fast grasshoppers.

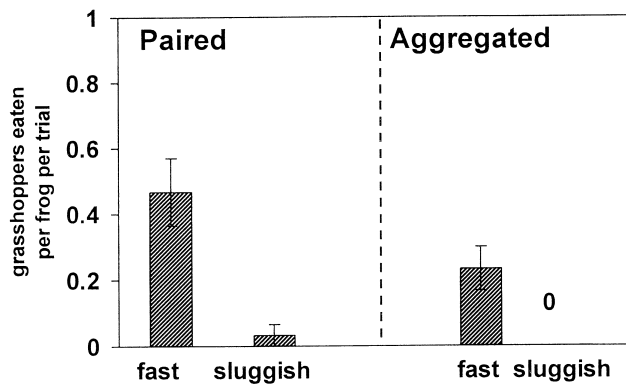


Figure 5. Survivorship (mean \pm SE) of sluggish- vs. fast-moving grasshoppers during encounters with frogs, in both paired and aggregated presentations. We offered grasshoppers to frogs as per Figure 1. In the paired trials, only two tethered grasshoppers were presented. In the aggregated trials, five untethered, sluggish grasshoppers were released between the two tethered grasshopper immediately before the frog was released. Within both paired and aggregated presentations, sluggish-moving grasshoppers had significantly higher survivorship than fast-moving grasshoppers. Within treatments and across presentations, there were no significant differences in survivorship for either sluggish or fast grasshoppers.

Table 1. Times (mean \pm SE in sec) for Northern leopard frogs to orient toward and snap at lubber grasshopper prey that had their rate of motion manipulated

	Paired		Aggregated	
	Fast	Sluggish	Fast	Sluggish
Orient	53.2 \pm 10.2 (15)	95 (1)	160 \pm 20.5 (8)	(0)
Snap	55.6 \pm 10.7 (14)	105 (1)	184 \pm 19.4 (7)	(0)

Lubbers are naturally sluggish-moving. Grasshoppers were presented as a choice test, either in a pairing (i.e., only two grasshoppers presented) or in an aggregation, with a total of seven grasshoppers presented. Each test had one fast grasshopper, which was induced with motion, and one sluggish grasshopper, which was allowed to exhibit its natural, slow behavior (see Fig. 1). Numbers in parentheses are the number of frog responses for 15 frogs, each offered every prey presentation twice (maximum possible = 30).

Discussion

Our results demonstrate that the fastest-moving individual in an aggregation of sluggish, aposematic insects is more likely to be attacked by a motion-oriented predator than its cohorts (Figs. 4 and 5). We also show that there exists repeatable variation in movement rate of sluggish prey on which natural selection could act (Fig. 2). Further, survivorship in encounters with motion-oriented predators was inversely related to the rate of prey movement (Fig. 3). Individuals in an aggregation are constantly competing against their cohorts to avoid predators (e.g., Hamilton, 1971; Vulinec, 1990). Taken together, our data imply that the survival disadvantage for fast-moving individuals in an aggregation could create a selection pressure for the evolution of sluggish movement as a defense in aposematic, chemically defended, arthropod prey. This is a realistic scenario by which defensive, sluggish movement could have evolved as an adaptation. It serves as an alternative hypothesis to the idea that sluggish movement in aposematic, chemically defended, gregarious insects evolved to save energy (implied in Chai and Srygley, 1990; Srygley and Chai, 1990a, b; Marden and Chai, 1991).

Variability exists in movement rate of third instar grasshoppers

Repeatable variability in grasshopper rate of movement was present in spite of the uniform environment of laboratory rearing (Fig. 2). Natural selection could act on this variability in movement rate. The directionality of insect movement is heritable (Ricker and Hirsch, 1988; Stoltenberg *et al.*, 1995; Plomin, 1997); it seems possible that the rate of insect movement is also heritable. We did not test whether movement rate in lubber grasshoppers was heritable, largely because of their 9-month generation time (Chladny and Whitman, 1997). Heritability of movement rates would be more readily tested

using an insect with a shorter generation time. We hypothesize that artificial selection for some slower individuals and other faster individuals within an aposematic species will produce differences in the defense of these cohorts from frogs.

Sluggish grasshoppers had higher survivorship than fast-moving grasshoppers

Sluggish grasshoppers survived significantly more trials than fast-moving grasshoppers, in encounters with frogs. These data, using a choice-test design, echo the results of Hatle and Faragher (1998) and suggest that sluggish movement can have defensive benefits against motion-oriented predators. The selective nature of these predators in choosing prey makes this defense possible. According to Roth (1986), the common assumption, that amphibians (which are the classic vertebrate motion-oriented predator) attack everything that is the right size and moves, is incorrect. Instead, motion-oriented predators can be quite discriminating and refuse to attack slow prey. For many predators, attack rate is positively correlated with rate of prey movement, up to certain limits (Roth, 1986; Hatle and Whitman, 2001). For example, Freed (1984) offered five different species of insect prey to the jumping spider *Phidippus audax*. Prey mortality mirrored prey velocity; slower-moving prey were killed less often than faster-moving prey. These results suggested that a certain level of prey velocity (2–5 cm/sec) was the best releaser of spider attacks. We also found that non-moving grasshoppers were attacked significantly less than sluggish-moving grasshoppers (Fig. 3). This suggests that a slower rate of movement than that exhibited by extant grasshoppers could be more beneficial. Our conclusion that slower movement would be more beneficial agrees with a wealth of previous research on the predatory behavior of motion-oriented predators (e.g., Kaufman, 1974; Luthardt and Roth, 1979; Anderson, 1993).

If remaining motionless has defensive benefits, why then do sluggish prey move at all? Most likely they move because of trade-offs between defense and other needs, such as foraging (Bernays *et al.*, 1992), mating, and thermoregulation (Whitman, 1987, 1988b). In addition, some sluggish movement in aposematic prey may actually have defensive benefits against predators if it increases the conspicuous nature of the insect and advertises unpalatability (Guilford, 1986, 1990; Srygley, 1994; Hatle and Whitman, 2001).

In aggregations, sluggish-moving prey had higher survivorship than fast-moving prey

The advantage of sluggish movement exists for aggregated prey as well as paired prey (Figs. 4 and 5). Our results demonstrate that aggregated individuals moving faster than their cohorts can suffer significantly higher mortality in

encounters with motion-oriented predators. Our model requires multiple prey so that relative rates of movement impart defensive benefits. The survivorship of sluggish individuals must merely be greater than the survival of faster cohorts, and this result was clear in all our experiments.

In comparing paired prey and aggregated prey, sluggish movement was not significantly more beneficial in aggregations (Table 1). This may be due in part to the already low attack rates on sluggish prey in paired presentations; in experiment 5, frogs ate only one sluggish prey in paired presentations and zero sluggish prey in aggregated presentations (Fig. 5). In contrast, fast-moving grasshoppers in an aggregation were attacked significantly later than fast-moving grasshoppers in a pairing (Table 1; $p < 0.0001$). In nature, such a delay could increase prey survivorship by allowing prey to escape. This result is consistent with the hypothesis that aggregation in aposematic prey can produce a greater, more alarming aposematic signal, which delays predator attack. This has been shown previously using both searching predators (birds; Sillén-Tullberg, 1990; Gamberale and Tullberg, 1996, 1998) and ambush predators (frogs; Hatle and Salazar, 2001). Our present results suggest that fast movement may be more detrimental in a small group than in a large group. We hypothesize that a trade-off exists between the benefits of a stronger aposematic signal (which is greater in a large group) and the benefits of sluggish movement (which may be greater in a small group).

Interestingly, the selection pressure against the fastest-moving individual may be strongest when the entire group is moving, rather than when most of the individuals in the group are nearly non-moving. Wilson *et al.* (1990) found that anti-apostatic selection against atypical color morphs was strongest at higher temperatures. The only difference between these high temperature trials and similar lower temperature trials appeared to be the rate of movement of individuals in the aggregation, which was faster at higher temperatures. The strongest selection pressure might exist when the fastest individuals are moving ~ 1 Hz (the rate that most strongly released toad attacks in Borchers *et al.*, 1978) and decrease as the fastest-moving individuals move slower.

The evolution of sluggish movement as a defense in aposematic prey

Competition for defense from motion-oriented predators within an aggregation may make possible the adaptive evolution of sluggish movement as a defense by small, incremental steps. Individuals in aggregations may compete to avoid predators (Hamilton, 1971). In our model, they do so by moving slower than their cohorts. Hence, in our model, relative (not absolute) rates of motion are important. Provided that relatively faster movement is detrimental, an evolutionary pressure to move slowly could be maintained by motion-oriented predators culling the fastest moving prey from each generation. Given

what is known about the prey characteristics that release amphibian attack (Roth, 1986; Ewert, 1987), this seems likely, up to a point. Slower rates of movement are progressively less likely to release attack. Hence, it is not surprising that prey, even conspicuous prey, might avoid predator attack relative to their cohorts by moving slower than the speed at which attacks are optimally released.

Sluggish movement as a defense may be particularly likely to evolve in chemically defended, aposematic, aggregated prey. Sluggish prey would be easy targets for seek-and-find predators (e.g., birds). Without some other deterrent such as chemical defense, it seems likely sluggish prey would be preferentially attacked. Further, researchers have often posited that gregarious behavior is advantageous because predators become satiated and some gregarious prey survive uneaten (Järvi *et al.*, 1981; Wiklund and Järvi, 1982; Turner and Pitcher, 1986; Sillén-Tullberg and Leimar, 1988). Chemical defense in gregarious prey would shorten the time needed for predators to learn to refuse further prey (Whitman *et al.*, 1990), especially if the prey is aposematically colored (Gittleman and Harvey, 1980; Roper and Wistow, 1986). After the faster-moving prey is eaten first, sluggish-moving individuals may survive when the predator becomes repelled by chemical defense, satiation, or deterred in any other way.

Defensive sluggish movement in aggregated prey: alternative hypotheses

We hypothesize that sluggish movement in aposematic, chemically defended, gregarious insects could have evolved as a defense in response to pressure from motion-oriented predators. The results presented in this paper are consistent with this hypothesis. However, our results do not eliminate the possibility of sluggish movement evolving through other routes, such as energy savings (Chai and Srygley, 1990), reducing recognition errors by predators (Guilford, 1986; Srygley, 1994), or maintaining cohesion of the aggregation. In this light, sluggish movement would serve as a defensive exaptation (Gould and Vrba, 1982; Ketterson and Nolan, 1999). Could sluggish behavior, seemingly maladaptive in encounters with predators, have actually evolved as a defense? Comparative experiments can address whether a particular behavior is an adaptation or exadaptation. Our model posits that sluggish movement is a possible outcome of gregarious living for arthropods. It predicts that aposematic, gregarious prey are often sluggish-moving, but that aposematic, sluggish-moving prey that are not gregarious would be less common. This prediction could be tested using a phylogenetic method (e.g., Johnson *et al.*, 2000). A robust test would require measuring the rates of movement of all the aposematic species (both solitary and gregarious) in a clade. We predict that gregarious species would, on average, move significantly more slowly than solitary species.

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