

Motion level in *Graphosoma lineatum* coincides with ontogenetic change in defensive colouration

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Abstract

Crypsis and aposematism are two different approaches to predation avoidance. Theoretically, the chosen strategy depends on the prevailing possibilities and constraints to the animal. Consequently, insects often change strategy between life stages, but a switch in strategy within a life stage is quite uncommon. In the Swedish shieldbug, *Graphosoma lineatum* L. (Heteroptera: Pentatomidae), there is a colour change from the pre-hibernating to the post-hibernating adults that alters their protective strategy from effective crypsis to aposematism, although both stages are distasteful to predators. The change in colour occurs as there is a shift of seasons, which alters the environmental background. Here, we investigate whether there is a change in behaviour in *G. lineatum* connected to the change in colouration and protective strategy. We therefore measured the motion levels in the two different colour forms of *G. lineatum*, both in the field and in a more controlled laboratory set up. Our result clearly shows that there is a change in behaviour connected to the change in colour. Thus, we found that the cryptic pre-hibernation form has a significantly lower motion level than the aposematic post-hibernation form, which is in line with the fact that motion disrupts crypsis, but not aposematism.

Introduction

Crypsis and aposematism are two different approaches to predation avoidance. Theoretically, the chosen strategy depends on the prevailing possibilities and constraints to the animal which, in turn, may depend on factors such as degree of unpalatability, life stage, size, mobility, vulnerability, and feeding rate (Merilaita & Tullberg, 2005; Speed & Ruxton, 2005; Higginson & Ruxton, 2010). Unprofitable prey often use a conspicuous aposematic colouration to deter attacks from predators when their vulnerability is relatively low and the risk of exposure is high (Poulton, 1890; Ruxton et al., 2004). Using cryptic colouration, on the other hand, is more common when vulnerability is high and when the risk of exposure, due to, for instance, motion, is relatively low (Cott, 1940). Experimental studies have shown that moving is detrimental to a cryptic strategy, as it attracts predators' attention and increases predation risk (Martel & Dill, 1995; Ioannou & Krause,

2009). Theoretical studies suggest that aposematic colouration is more likely to evolve when constraints of crypsis are too high. (Merilaita & Tullberg, 2005; Speed & Ruxton, 2005). According to these theories, aposematic individuals are freer to move around while cryptic individuals need to stay motionless not to be detected and eaten. Thus, if all else is equal, there should be a general correlation between crypsis and low activity on the one hand, and aposematic signalling and high activity on the other.

Insects often change strategy depending on the life stage they are in (see Booth, 1990 for a review). Some Lepidoptera use crypsis during their youngest instars and as immobilised pupae, but change to an aposematic strategy as larger larvae and in the adult life stage (Wiklund & Silken-Tullberg, 1985). A change in colouration within the same instar is not as common (Booth, 1990), but when it occurs, it is often used to maintain a cryptic function in a changing environment (Chapman, 1998). However, in Swedish populations of the striated shield bug, *Graphosoma lineatum* L. (Heteroptera: Pentatomidae), there is an irreversible colour change from the pre-hibernating to the post-hibernating adults. This colour change alters the

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protective strategy from effective crypsis to aposematism (Johansen et al., 2010), although both stages are distasteful to predators (Gamberale-Stille et al., 2010). The pale form goes into hibernation hidden in the undergrowth and changes its colour to red before it emerges in spring. The change in colour occurs as there is a shift of environmental background: the cryptic, pre-hibernating shield bug lives in a dry, brownish late-summer environment whereas the aposematic post-hibernating bug is seen against a lush green late-spring background.

In this study, we aimed to investigate if there is a change in behaviour in *G. lineatum* coinciding with the change in colouration and protective strategy. We therefore measured the motion level of the bugs during different seasons, both in the field and under controlled laboratory conditions. Specifically, we tested the hypothesis that the level of motion/activity is lower in the pale pre-hibernating stage than in the red post-hibernating stage.

Materials and methods

Field observations

The striated shield bug, *G. lineatum* (see Johansen et al., 2010 for a detailed description of the biology), feeds on plants and seeds of various species of Apiaceae in our study area, mainly *Anthriscus sylvestris* (L.) Hoffm. and *Aegopodium podagraria* L. *Graphosoma lineatum* uses its piercing sucking mouthparts to feed on ovula and seeds that are still attached to the plant and it finds a position allowing it to remain motionless while feeding (AI Johansen, pers. obs.). Three locations in the Stockholm area [Aspudden (59.31°N, 17.99°E), Haga (59.35°N, 18.04°E), and Stora Skuggan (59.36°N, 18.06°E)], each with several patches of the host plants, were sampled on four different occasions in 2007: red post-hibernation adults on 12 and 26 June, and pale (new generation) pre-hibernation adults on 11 and 26 August. Time of day, temperature, and activity was noted for a total of 455 individual observations. The number of individuals in a patch was noted and, for every observed animal, a behavioural score (an instantaneous point sample) was taken at the same time. We noted if the individual was moving or sitting still. We also noted where it was sitting, if it was feeding (by observing the mouth parts), walking, flying, or copulating. Feeding and copulating were classified as not moving, whereas flying was included in the moving category. We did not identify the sex of the animals, as that would have disturbed them. We wanted to investigate whether there were any behavioural differences between the newly eclosed pale stage and the reproductive red stage of the shield bugs. Therefore, we compared the behaviour of red bugs with the behaviour of the pale bugs 2 months apart. We compared red bugs sam-

pled on 12 June with pale bugs sampled on 11 August in one test. Furthermore, we compared red bugs sampled on 26 June with pale bugs sampled on 26 August in a second test to see if the result was repeatable. In total, we observed 111 red bugs (35 and 76 for the two observation dates, respectively), and 326 pale bugs (211 and 115 for the two observation dates, respectively).

Laboratory observations

To be able to control for differences between the sexes and for the influence of temperature and environment on the behaviour of the bugs, we conducted a laboratory experiment. *Graphosoma lineatum* was collected from the field during two time periods in 2009, in June and August, coinciding with the shift in generation and colour in the field. Bugs were kept for a maximum of 3 days, in plastic terrariums under conditions similar to those in the field, to minimise the effect on their activity of being kept in a confined space. We used three cages (0.8 × 0.8 × 0.5 m), with fabric walls, a clear plastic ceiling and one wall of mesh that could be opened and used to see through. Cages were each equipped with four commercial, organically grown potted parsley plants and we added dry seeded umbels from the host plant *A. sylvestris* on a small wooden stick in each pot. In each cage, we placed four *G. lineatum* individuals, two females and two males, individually marked with black Penol permanent marker pens, one on each parsley plant in the experimental cage. This was done at 10:40 hours, after which we waited 30 min before starting to take recordings continuously for 5 min. This was repeated every half-hour, resulting in seven measurements for each individual. The temperature was noted for each recording period. At each observation, we continuously recorded the location of each individual bug, to find out whether it had moved since the last recording. Furthermore, we continuously observed whether the bugs were moving about or stayed still throughout the 5 min of observation time, and we noted if they were flying, copulating, walking, or sitting still. Copulating was classified as sitting still while flying was classified as moving. We did not distinguish between if a bug was just sitting still or also feeding because that would not have been possible without disturbing the bugs in the small space. To quantify the motion level, we assigned points to each observation; 0 if a bug was sitting still or had not moved since the last observation and 1 point if the bug was moving or had moved since the last observation. Individual bugs were given a motion level with the minimum value of 0 for the ones that did not move at all, and a maximum of seven for those that moved in all measurements. A total of 48 red bugs (24 males and 24 females) were observed from 2 to 10 June, and a total of 72 pale bugs (35 males and 37 females) were observed from

11 to 20 August (the uneven numbers of pale males and females is explained by one female being mistaken for a male during the experiment, but then treated as female in the analyses). All statistics were performed in R 2.12.2. (Ihaka & Gentleman, 1996), and only non-parametric tests (Fisher's Exact Test and Mann-Whitney U-test) were used.

Results and discussion

On two field observation dates in June, we observed a total of four shield bugs in flight and 24 pairs in copula (Table 1), whereas none of these behaviours was observed in August. In total, when comparing the proportion of individuals moving or sitting still in the field, the red shield bugs (June) had a higher motion level than the pale ones (August) in the two comparisons (Table 1; 12 June vs. 11 August and 26 June vs. 26 August; both Fisher's Exact Test: $P < 0.0001$). Also, among the non-moving individuals, a much higher proportion was found feeding in the samples from pre-hibernating than from post-hibernating bugs (Table 1; 12 June vs. 11 August and 26 June vs. 26 August; both Fisher's Exact Test: $P < 0.0001$). As the pale bugs are newly moulted adults facing a long period of hibernation, their main need is to forage.

In the laboratory, we observed 17 individuals flying (Table 2), all of which were red post-hibernation bugs (red vs. pale, Fisher's Exact Test: $P < 0.0001$). Of the flying red bugs in the laboratory observations, there were more males than females (Fisher's Exact Test: $P = 0.015$). Also, three pairs of red bugs were seen in copula (Table 2). There was a clear difference in the mean motion level (see Materials and methods) between red and pale shield bugs, where the red bugs moved around significantly more

Table 1 Number of *Graphosoma lineatum* individuals observed not moving, whereof the number of feeding or copulating individuals within parenthesis, and individuals observed moving, whereof the number in flight within parenthesis, at four occasions in 2007 in the field

Date	Temperature (°C)	n	Not moving	
			(feeding, in copula)	Moving (flying)
12 June	24	35	23 (0, 6)	12 (1)
26 June	30	76	43 (12, 18)	33 (3)
11 August	25	55	53 (44, 0)	2 (0)
26 August	16	87	84 (82, 0)	3 (0)

Red shield bugs belong to the post-hibernation phase at 12 and 26 June, and pale shield bugs belong to the new pre-hibernation phase at 11 and 26 August. See text for statistical analysis.

Table 2 Mean (\pm SE) motion levels of male and female *Graphosoma lineatum* in the red post-hibernation and pale pre-hibernation stage

Colour	Sex	n	Mean motion		
			level (0–7) \pm SE	In copula	Flying
Red	Male	24	5.9 \pm 0.18a	3	13
Red	Female	24	3.9 \pm 0.31b	3	4
Pale	Male	35	3.2 \pm 0.38bc	0	0
Pale	Female	37	2.7 \pm 0.38c	0	0

Minimum = 0, maximum = 7. Temperature = 26 ± 1 °C. Means followed by different letters are significantly different (Mann-Whitney U-test: $P < 0.05$).

(Table 2; Mann-Whitney U-test: $P < 0.0001$). In a comparison between the sexes, the males of the red form moved about significantly more than the red females (Table 2; Mann-Whitney U-test: $P < 0.0001$), whereas there was no significant difference between pale males and females (Table 2; Mann-Whitney U-test: $P = 0.34$). Also, the red shield bug females moved around more than both the pale females (Table 2; Mann-Whitney U-test: $P = 0.023$) and the pale males (Table 2; Mann-Whitney U-test: $P < 0.0001$).

In the laboratory experiment, we found that red males had the highest motion levels of all bugs and moved significantly more than red females, whereas we found no difference in motion level between the sexes in the pale non-reproductive form. The high activity of the red males is likely due to an active search for receptive females (see for instance Maes et al., 2006; Karlsson & Johansson, 2008). However, we found that reproductive red females also move around more than the pre-hibernation pale females, possibly due to the need to search for good oviposition sites and to avoid sexual harassment (e.g., Shuker et al., 2006). Although our field observations are not directly comparable to the laboratory study because of the differences in methods used to score activity level, our laboratory study indicates that the increased activity found in the reproductive bugs in the field is due to an increase in both sexes, but with a more pronounced increase in activity level in the males.

These results clearly show that there is a change in behaviour coinciding with the change in colour and adult stage. Both the field and the laboratory observations show a significant difference, with a high motion level in the red shield bugs and a low motion level in pale shield bugs. These differences cannot be artefacts of, for instance, temperature, the quality of the environment, or senescence of the bugs, as we kept the temperature (26 °C) and the environment the same in the laboratory, and it is the

older red individuals that move around more. It is far more likely that there is a selective reason for the correlation between the respective adult life stage and its colouration.

As mentioned, there are several likely factors that may influence the cost/benefit ratio of different primary defence strategies, one major factor being the great opportunity costs that a cryptic strategy entails because of the need to stay motionless (Merilaita & Tullberg, 2005; Speed & Ruxton, 2005). As the lined shield bug may feed for long periods without movement, such costs are greatly reduced for the pre-hibernating but not for the post-hibernating reproductive stage. Moreover, experimental studies that compare the detectability (when motionless) of the two colour forms in late-summer environments show a great advantage of the pale colouration in terms of decreased detection risk (Tullberg et al., 2008; Johansen et al., 2010). These factors may be of major importance to explain the selective advantage of crypsis in the newly eclosed adults. The red post-hibernation colour form, on the other hand, has been shown to invoke more averseness on sight in birds than the pale form (Gamberale-Stille et al., 2010). Bugs in the red colour form are also in a reproductive state, which demands a higher degree of activity, which would greatly raise the costs of crypsis compared with the pre-hibernation form. These factors may thus be of importance in explaining why the bug does not retain its pale colour, but switches to a red aposematic colour in the spring.

Both the pale and the red form of *G. lineatum* are well defended. Both have a very hard cuticula and emit defensive substances when threatened (Stránský et al., 1998; Gamberale-Stille et al., 2010). It is not known, however, if the forms differ in distastefulness, but a small bio-assay of palatability to domestic chicks did not show any difference between them (Tullberg et al., 2008). Nevertheless, the possibility remains that the diet composition of the bugs may vary over the year, and if their defences are somewhat dependent on diet, potential differences in chemical defence between the forms cannot be ruled out at this point.

Another factor that may influence the variable use of defence strategy in these bugs is that the predation pressure from insectivorous bird species may change over the seasons. For instance, predation pressure may be lower in late spring and summer when there are plenty of caterpillars and other more preferred prey available and higher in autumn when the numbers of the preferred prey decline (Ide, 2006). At the same time, there is a change in the predator community. This change involves a shift from more experienced bird predators in spring, to an increase in inexperienced predators in late summer, when yearling

birds need to fend for themselves and learn about prey profitability (Svádová et al., 2009). From a prey's perspective, it might be a better strategy to avoid detection altogether because the risk of being sampled is high even if the individual is aposematic. This would further benefit the pale strategy of *G. lineatum* in autumn. Also, when red aposematic *G. lineatum* emerges in spring it appears later than other aposematic red and black species such as the ladybird beetle *Coccinella septempunctata* L, the fire bug *Pyrrhocoris apterus* L. and the seed bug *Lygaeus equestris* (Species Gateway, 2010). It has been shown that birds do generalise between these species of red and black aposematic bugs to a greater or lesser extent depending on the bird species (Svádová et al., 2010). Late emergence may further reduce predation on aposematic *G. lineatum* in summer.

We do not claim that these results show causality between the colour change and levels of mobility. However, we conclude that there are many, not mutually exclusive, benefits to changing defensive colouration with season in *G. lineatum*, and that this study shows that the behaviour of *G. lineatum* is in accordance with expectations from the defensive colouration theory. In Sweden, individuals of this species show a more cryptic colour pattern at times when cost of reduced activity is low, which corresponds to low motion levels. Likewise, they adopt a signalling strategy when the opportunity costs of crypsis are high and when they have high motion levels.

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