Adaptive change in protective coloration in adult striated shieldbugs *Graphosoma lineatum* (Heteroptera: Pentatomidae): test of detectability of two colour forms by avian predators

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Abstract. 1. Protective coloration in insects may be aposematic or cryptic, and some species change defensive strategy between instars. In Sweden, the adult striated shieldbugs *Graphosoma lineatum* (Heteroptera: Pentatomidae) undergo a seasonal colour change from pale brown and black striation in the pre-hibernating adults, to red and black striation in the same post-hibernating individuals. To the human eye the pre-hibernating adults appear cryptic against the withered late summer vegetation, whereas the red and black post-hibernating adults appear aposematic. This suggests a possibility of a functional colour change. However, what is cryptic to the human eye is not necessarily cryptic to a potential predator.

2. Therefore we tested the effect of coloration in adult *G. lineatum* on their detectability for avian predators. Great tits (*Parus major*) were trained to eat sunflower seeds hidden inside the emptied exoskeletons of pale or red *G. lineatum*. Then the detection time for both colour forms was measured in a dry vegetation environment.

3. The birds required a longer time to find the pale form of *G. lineatum* than the red one. The pale form appears more cryptic on withered late summer vegetation than the red form, not only to the human eye but also to avian predators. The result supports the idea that the adult individuals of *G. lineatum* undergo a functional change from a cryptic protective coloration to an aposematic one.

Key words. Antipredatory defence, aposematism, background matching, crypsis, great tit, ontogenetic colour change, *Parus major,* warning coloration.

Introduction

Protective coloration may be crudely divided into aposematic (warning) coloration and camouflage (crypsis). Warning coloration is used to deter attacks from predators by advertising unpalatability (Poulton, 1890; Ruxton *et al.*, 2004) or to deceive predators by false signalling (Batesian mimicry). The function of crypsis is to avoid attack by avoiding detection altogether (Cott, 1940; Edmunds, 1974). In theory this seems straightforward: poisonous, unpalatable, or otherwise protected species with conspicuous coloration are aposematic whereas unprotected species are camouflaged for instance by background matching.

However, there are species that employ both types of protective strategies and change from one to the other in different instars or life stages. Such an ontogenetic colour change involving a switch from cryptic to aposematic coloration between different instars is not uncommon in insects (see Booth, 1990 for review). For example, caterpillars of younger instars of some lepidopteran species are cryptic, whereas older instars are aposematic (Grant, 2007); the adult and the larva of both the monarch butterfly (*Danaus*

plexippus) and the European swallowtail (*Papilio machaon*) are aposematic, whereas the pupa is cryptic (Wiklund & Sillén-Tullberg, 1985). Changes in host plant, mobility, vulnerability, size, and feeding rate between different instars can alter the relative costs and benefits of camouflage and aposematic coloration (Merilaita & Tullberg, 2005; Speed & Ruxton, 2005; Grant, 2007; Higginson & Ruxton, 2010).

In contrast to the frequent occurrence of ontogenetic colour change taking place in successive instars, a change in coloration within the same instar is less common (Booth, 1990). In many cryptic species of true bugs (Heteroptera) the adult post-moult process of melanization is irreversible and extends over the whole adult period (e.g. many Miridae). In others, such as *Plautia stali, Nezara viridula*, and *Palomena* spp (Pentatomidae), the adult undergoes a reversible colour change between green and brown, which is associated with hibernation and a continuous cryptic function (Kotaki, 1998; Musolin & Numata, 2003; P. Štys, unpublished). Chapman (1998) summarised similar reversible intra-instar colour changes occurring in other insects (e.g. Auchenorrhyncha, Orthoptera, and Neuroptera) associated with a change of season or environment. However, these examples all seem to involve changes between functionally similar colorations and, to our knowledge, no case has previously been documented of a functional colour change from cryptic to aposematic coloration within the same instar.

In Swedish populations of the striated shieldbug, *Graphosoma lineatum* (Heteroptera: Pentatomidae), the adult undergoes a potentially adaptive and definitely irreversible colour change. The newly eclosed pre-hibernating adults exhibit a pale brown and black striation which changes to a conspicuously red and black striation during hibernation (Tullberg *et al.*, 2008). This colour change is associated with a seasonal change of vegetation; the pale brown colour occurring among withered late summer plants, and the red colour among lush green spring and summer vegetation.

We suggest that this is an adaptive intra-instar ontogenetic colour change, and that the bug has a more cryptic life style in late summer and changes to a predominantly aposematic life style after hibernation. In support of this there is experimental evidence that birds show a greater aversion for the red than for the pale form (Gamberale-Stille *et al.*, 2010). We expect that the pale form is harder to detect than the red form against a late summer background and in a photographic image experiment using humans as predators, this was indeed found to be the case (Tullberg *et al.*, 2008). However, the visual system of birds differs from that of humans in several aspects, mainly in the perception of colours (Bowmaker, 2004) and in visual acuity (Donner, 1951; Güntürkün, 2000; Yamamoto *et al.*, 2001), both of which could influence the prey detection efficiency. Thus, a more reliable test of difference in detection risk in *G. lineatum* would come from experiments using its potential predators, such as insectivorous birds.

Therefore, in the present study we tested whether the two forms of *G. lineatum* differ in their detectability for an avian predator in a semi-natural environment of dry vegetation. As predators we used two experimental groups of wild-caught great tits (*Parus major*), one of them pre-trained, and tested with pale *G. lineatum* baits, and the other with red ones.

Materials and methods

Biology of Graphosoma lineatum

The striated shieldbug *G. lineatum* (L. 1758) (Heteroptera: Pentatomidae) is a common European phytophagous true bug, and it produces noxious semiochemicals (Stránský *et al.*, 1998) in its metathoracic (adult) or dorsal abdominal (larva) scent glands. Adult shieldbugs are avoided by most passerines, including wild caught great tits (Veselý *et al.*, 2006; Exnerová *et al.*, 2008; Hotová Svádová *et al.*, 2010). The striated shieldbug is associated with host plants of the family Apiaceae, in Sweden mainly *Anthriscus sylvestris* and *Aegopodium podagraria*. In the northern part of its distribution it is univoltine and the adult individuals exhibit two successive colour forms. There are five larval instars (Fig. 1), all cryptically coloured in various shades of brown and black. In late summer the fifth instar larvae moult into prehibernating adults that have a pale brown and black striation (Fig. 2a), which appears cryptic to the human eye against the background of dry late summer vegetation (Tullberg *et al.*, 2008). These new generation pale adults spend most of their time feeding motionless on seeds of their host plants. In autumn the pale brown and black striated adults enter hibernation, however, when the same individuals emerge in spring they exhibit a brightly red and black striation (Fig. 2a). The change in coloration is effected by a change in epidermal coloration whereas the black melanised cuticular stripes remain unchanged between the seasons (Tietz & Zrzavy, 1996). The red and black post-hibernation adults are reproductively active, moving around locating mates and oviposition sites in a fresh green environment, as well as feeding from the flowering (white) umbels of their host plants (A. I. Johansen *et al.*, unpublished). During spring and early summer they mate and oviposit several times before they die. The colour change described above probably represents a derived condition typical for the populations of *G. lineatum* close to the northern boundary for the species. In populations living in Central Europe (and Mediterranean), seasonal colour polyphenism exists. Individuals that moult into an adult stage early in the season are red and black, whereas those that moult in the late summer possess the pale brown and black coloration (P. Štys *et al.*, unpublished). However, in these areas too, all post-hibernation adults are red-and-black.

Experiment

Predators

We used great tits (*Parus major*), a frequently used model predator in ethological studies. We considered great tits particularly suitable for our purpose because they show a higher willingness to attack unfamiliar prey than other European tit species, and avoidance of aposematic prey in young naïve great tits is to a large extent based on learning (Exnerová *et al.*, 2007). The great tit is a small arboreal passerine living in all kinds of woodland, often in close proximity to human settlements and in parks and gardens,
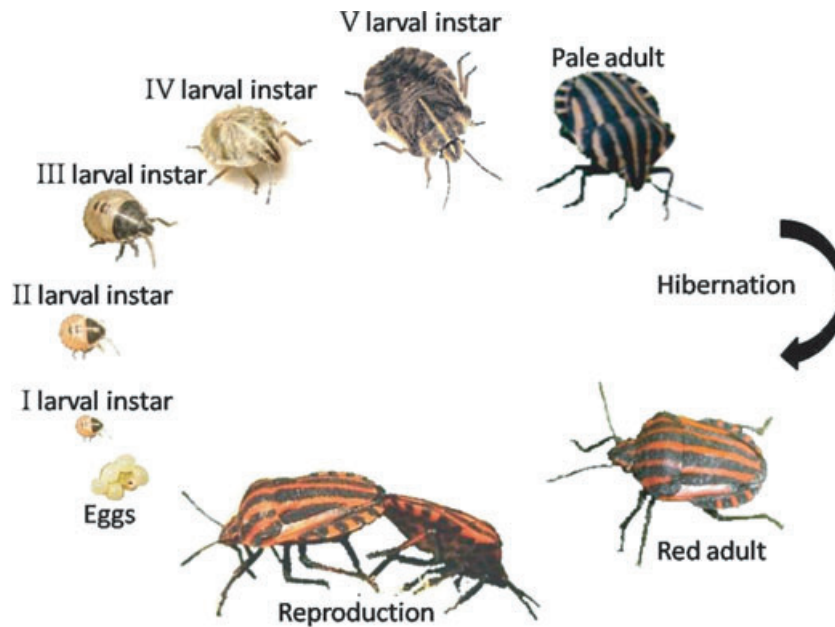
Fig. 1. Life cycle of *Graphosoma lineatum* in Sweden. It has five larval instars with a soft exoskeleton. In late summer the fifth larval instar moults into a pale and black striated adult with a fully sclerotised hard exoskeleton; it spends most of its time feeding motionless on seeds. The pale adults enter hibernation in late autumn and emerge at the end of May having changed their colour to red and black. These red and black spring adults are reproductively mature, moving around locating mates and oviposition sites.

feeding mainly on insects, seeds, fruits, and berries (Cramp & Perrins, 1993; Gidstam & Wahlin, 2007). The experiment, using great tits as predators, was carried out under permit from Linköpings djurförsöksetiska nämnd (DNr. 16-05), and was conducted in February 2008 at Tovetorp Zoological Research Station, 90 km south of Stockholm, Sweden. Great tits were caught with mist-nets and housed individually in cages (housing cages: 80 × 60 × 40 cm) with water, sunflower seeds, and suet balls *ad libitum*. Cages were equipped with two perches for resting. Birds were kept for 2–3 days; on the day of capture they were trained to handle baits, the second day they participated in the experiment, and the following morning they were ringed and released at the site of capture.

Baits

To test the detectability of aposematic prey for wild-caught birds is problematic, because their previous experience with such prey is unknown. If a bird has learned to avoid unpalatable insects of a certain appearance, it is likely to avoid such a prey upon discovery, this renders it impossible to determine insect detectability. To overcome this predicament we used baits made of dried specimens of *G. lineatum* and sunflower seeds. We trained the birds with these baits prior to the experiment to give them a positive experience of *G. lineatum* baits as a source of palatable food.

G. lineatum was reared on parsley (*Petroselinum crispum*) and seeds of *Aegopodium podagraria* and *Anthriscus sylvestris* in laboratory cultures at a light/dark regime of LD 12:12 h and 26 °C. The diapause of Swedish *G. lineatum* can be broken by

keeping the bugs at 26 °C for 2 weeks, whereupon they will change colour from pale to red and become reproductively active. For the preparation of baits for this experiment, about half of the adult bugs were killed a few days after emergence, when they were still pale, whereas the other half were killed after being allowed to change to a red colour. All bugs were killed by freezing and thereafter dried in a warm room. We removed the legs, the ventral abdominal exoskeleton and all remnants of the soft internal parts of the abdomen (Fig. 2). The abdominal dorsum is attached to the wings and the bug retains its natural shape and colour patterns when viewed from above (Fig. 2a). A husked and halved sunflower seed was glued (Glue stick, non-toxic, Snopake Ref. No 11613) into the ventral side of the bug in place of its abdomen (Fig. 2b).

Training

The birds were assigned to one of two treatments, corresponding to the two colour forms, either the pale (hereafter 'pale experimental group') or red ('red experimental group') *G. lineatum*. The sample size was 23 birds in each group. The birds were thus trained exclusively with one colour form of *G. lineatum*, and later tested with the same colour form of bait as in training. When trained the experimental groups were not significantly different in terms of the birds' sex (pale: 11♀/12♂, red: 10♀/13♂, Fisher's exact test, $P = 1$) and age (pale: 11 yearlings/12 adults, and red: 15 yearlings/8 adults, Fisher's exact test, $P = 0.373$). Furthermore the behaviour of yearlings and adults in the experiment was not significantly different in terms of mean search time (two sample *t*-test,

with Scotch tape at five different positions (Fig. 3a,b) identical for all birds and treatments. Birds had access to water *ad libitum* during the experiment.

Birds were tested individually and transported from the housing cages in a small transport cage, which was then
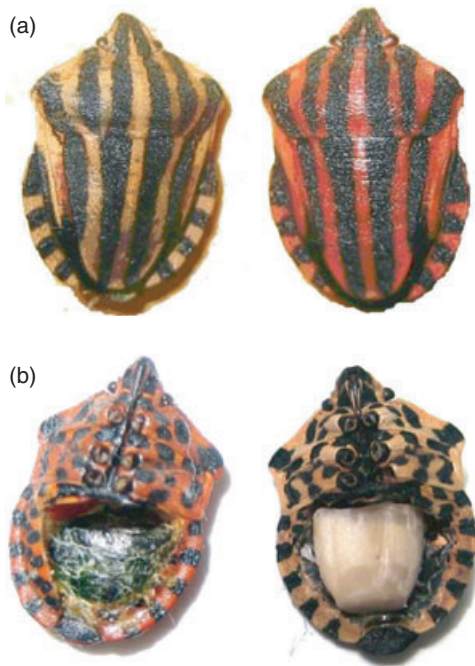

Fig. 2. (a) Pale (left) and red (right) adults of *Graphosoma lineatum* used in the experiment. (b) Red adults of *G. lineatum* with the ventral part of the abdomen removed (left) and pale *G. lineatum* with a glued sunflower seed (right).

$t = 0.097$, d.f. $= 44$, $P = 0.923$). To increase the birds' feeding motivation, they were deprived of food for 2 h before the training as well as before the experiment.

Birds were trained to handle the baits in their housing cages. First, in three consecutive trials the bait was presented with the ventral side up and the sunflower seed visible. After this, we presented the bait with the dorsal side up for an additional five consecutive trials. Latency of attacking the first bait and the total duration of the training time was measured to test for potential biases for one of the colours. Because the birds were trained the day before the experiment, they were offered two additional baits (dorsal side up) as reminders just before we moved them to the experimental room.

Experimental procedure

The experimental room (226 cm × 234 cm × 204 cm), with two one-way-glass windows (80 cm × 53.5 cm), a perch in one corner, and a log on the floor (Fig. 3a,b), was made of wood and plywood/MDF boards. It was lit by fluorescent Daylight lamps (Philips TLD 36W/965). The floor of the experimental room was covered with dry grass, stones, and dry herbaceous plants (*Hypericum* sp., *Achillea millefolium*, *Artemisia vulgaris*, and *Anthriscus sylvestris*). Some of the plants and grass were attached to stones and to the walls with Scotch tape (Fig. 3b), to make the environment more three-dimensional. Five umbels of *A. sylvestris* were prepared with one *G. lineatum* bait each (Fig. 3c) and attached to the floor
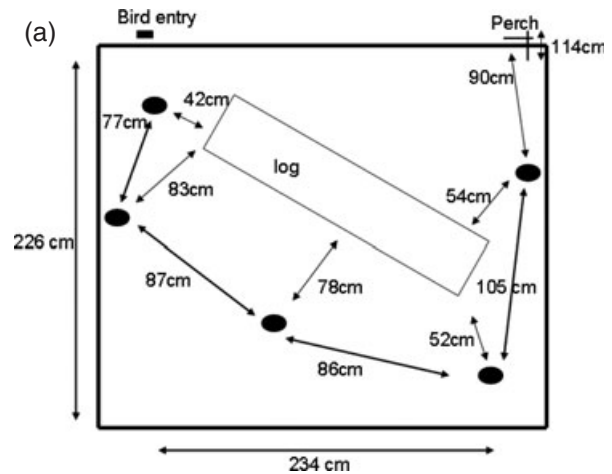

Fig. 3. Experimental setup. (a) Design of the experimental room. Distances in cm, black ellipses showing bait placement. (b) Experimental room. Bait position indicated by paper squares (not present during the experiment). (c) Pale (left) and red (right) *Graphosoma lineatum* glued onto the umbels of *Anthriscus sylvestris* in the experimental room.

connected to the wall of the experimental room against a small hole, and opened up. The test started when the bird left the transport cage and flew into the experimental room.

During the test, the bird was allowed to search for all of the five baits present. After the bird found and consumed its first bait, it was given 20 more minutes to search for and consume the other baits, and the test was stopped even if the bird had not found all five baits. Also, the test was stopped as soon as a bird had found and consumed all five baits within 20 min. If a bird did not find any of the baits within 30 min, we terminated the trial and the bird was excluded from the experiment: there were five birds that did not find any of the baits, two in the red group and three in the pale group. After the experiment, the birds were returned to their housing cages.

All experiments were recorded by video camera and bird behaviour was registered as a continuous record using the Observer Video-Pro 4.0 software (Noldus Information Technology, Wageningen, The Netherlands). Eight elements of bird behaviour were distinguished: flying, exploring (ground, log, and perch), drinking, eating the particular bait, handling the particular bait, passing close to the particular bait, resting, and sitting (ceiling, ground, log, and perch). Search time was defined as the time the birds spent actively exploring the experimental room before finding a bait (indicated by start of handling). Thus, the time spent handling and eating the previous baits, resting, flying, and drinking was excluded from the analyses.

Statistics

Most birds found the first four baits (83% pale, 78% red), whereas a much lower percentage found all five baits (52% pale, 65% red). Therefore, to maintain large sample sizes, as the ANOVA requires a balanced design, we excluded data for the fifth bait from the analysis and compared latencies only for birds that found four baits. All statistics were performed in R 2.6.2 (Ihaka & Gentleman, 1996), and all time data were log-transformed and tested with Bartlett's test of homogeneity of variances to make sure they fit the assumptions of parametric tests before t-tests or ANOVA were applied. We used a three factor mixed model ANOVA, where the latencies for the individual baits were the response variable, the experimental group (pale/red) and the order of bait discovery (1–4) were fixed factors, and bird individual was classified as a random factor. The 'latency' variable is referring to the effective search time for each of the baits found by the birds, excluding all other activities such as handling and eating a bait, resting, flying, and drinking. In addition to the ANOVA encompassing the latencies for four baits, the latencies for the first bait found were compared using a t-test, and the latencies for the second through fourth bait were compared in an ANOVA. The probability of each bait position to be discovered first was analysed using two-tailed Contingency tables (Fisher's exact probability test for 2×2 tables, and the Freeman–Halton extension of Fisher's exact probability test for 2×5 tables; Freeman & Halton, 1951).

Results

Training

There was no significant difference between birds receiving the pale and red *G. lineatum* bait in latency to attack the first training bait (pale/red: mean $\pm$ SD = 19.92 $\pm$ 19.10/18.68 $\pm$ 23.94 min, two sample t-test, $t = 0.194$, d.f. = 44, $P = 0.847$). Moreover, there was no difference in the total duration of the training time between birds trained with pale and red *G. lineatum* baits (pale/red: mean $\pm$ SD = 67.22 $\pm$ 29.25/60.04 $\pm$ 51.34, min, two sample t-test, t = 1.407, df = 44, $P = 0.167$). The mean latency in attacking the two reminder baits, which were given just prior to the experimental trial, did not differ between the two groups (two-sample t-test, $t = 0.320$, d.f. = 44, $P = 0.751$). As there was no difference between the groups in training time or latency in attacking the reminder baits offered just before the experiment, the groups were pooled together to test for the difference in time to attack between training and the reminder baits. The mean latency for baits in the training [total duration divided by number of training trials (eight)] was significantly longer than the mean latency of the two reminder baits given to the bird right before the experimental trial (two-sample t-test, $t = 20.670$, d.f. = 44, $P < 0.001$), suggesting a positive learning effect from training.

Experiments

The number of baits found by individual birds did not differ between the two experimental groups (two-sample t-test, $t = -0.856$, d.f. = 44, $P = 0.396$). However, the birds in the pale experimental group needed a significantly longer time to find their first bait compared with the birds in the red experimental group (two-sample t-test, $t = 3.52$, d.f. = 44, $P = 0.001$, Fig. 4).

Most birds found four baits (83% pale, 78% red) whereas a much lower percentage found all five baits (52% pale, 65% red). The latencies were significantly longer for birds in the pale experimental group than for birds in the red experimental group (three factor mixed model ANOVA; Table 1). The 'Within bird' measurement in Table 1 suggests that for the individual bird there is no significant change in latency between baits found, i.e. generally it did not get easier or harder for the individual birds to find the baits over the experiment.

It could be argued that the difference in latencies between the two experimental groups is solely as a result of the difference in latency for the first bait. However, if we exclude the first bait, latencies for the remaining baits are still significantly longer for the pale baits (three factor mixed model ANOVA: $F_{1,35} = 6.165$, $P = 0.018$).

There was no difference between the experimental groups in how long it took the birds to start exploring the ground in the experimental room (two-sample t-test, $t = -0.551$, d.f. = 44, $P = 0.584$). Individual birds seemed to apply different search strategies. Some birds jumped directly to the ground from the transport cage, which put them right next to the bait placed close to the bird entry (Fig. 3a,b) and then used the log as a place to handle and eat the baits. Others flew to the ceiling
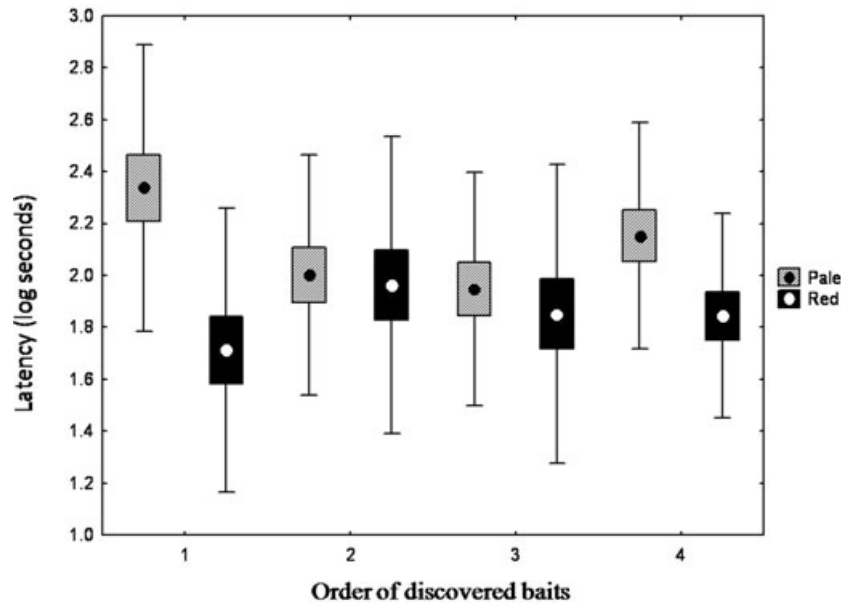
Fig. 4. Latencies (seconds log transformed) for birds to find the baits. Latencies are given separately for baits 1–4 and for experimental groups of birds offered pale (grey boxes) and red (black boxes) *Graphosoma lineatum* baits. Middle dot = mean, box = ±SE , whiskers = ±SD.

Table 1. Analysis of latencies for a bird's first, second, third, and fourth bait; only the data for birds that found all four baits are included. Results of three factor mixed model ANOVA, where the latencies for the individual baits were the response variable, the two experimental groups (pale/red) and the order of bait discovery (1–4) were fixed factors, and bird individual was classified as a random factor.

Source	df	MS	F	P
Experimental group	1	3.902	10.294	0.003[**]
Residuals	35	0.379	–	–
Within bird				
Latencies 1–4	3	0.225	1.045	0.376
Residuals	108	0.21	–	–

[**]Significant difference in latencies between the experimental groups. 'Within bird'- shows that the individual bird has no significant change in latencies between baits found.

and then settled on the perch using it as a base for feeding and scouting for baits, making other baits (Fig. 3a,b) easier to spot first. However, when comparing the frequency for each bait to be found first in the two experimental groups, there was no difference between them (Fisher's exact test, $P = 0.270$). We therefore pooled the two groups of birds together and looked at the probability of the baits in the five individual positions to be found first, but found no significant difference between bait positions (Chi-square goodness of fit $\chi^2_{0.05,4} = 4.434$, $P = 0.350$).

Discussion

The birds in our experiment took a longer time to find the pale form than the red form of *G. lineatum* in a dry semi-natural grass and herb environment. These findings strongly indicate that the pale form of *G. lineatum* is more cryptic for bird predators in its natural late summer environment than the red form. Moreover, the results are in accordance with the previous findings that the pale brown coloration of *G. lineatum* is more cryptic than its red coloration to human observers (Tullberg *et al.*, 2008). The latency to the start of exploration of the experimental environment did not differ between the two experimental groups of great tits. This lack of difference indicates that potential confounding factors, such as the level of stress or willingness to perform the task, were equal in the two groups. The fact that the detection time was the only parameter that differed between the two experimental groups makes our results quite robust.

Figure 4 suggests that the greatest difference in latency between the two colour forms occurs when searching for the first bait. Birds in the pale treatment seem to need a longer time to find the first bait as compared with the baits that followed. This suggests that the birds may have established a search image for the prey. Although this may have affected the search behaviour in our experiment it should not be an issue in nature. The forming of a search image is unlikely in the wild as the shieldbugs are chemically protected and there would not be a positive reinforcement facilitating its establishment.

There are several aspects of coloration in *G. lineatum* that may influence its detectability and might be responsible for the difference between the two forms: such as reflectance in UV, contrast with the background, internal contrasts, and disruptive effect. Tullberg *et al.* (2008) showed that the reflectance in the UV part of the light spectrum of both colour forms of *G. lineatum* and its dry late summer host plant (*A. sylvestris*) is very low, indicating that the ability of passerines to perceive UV-light is not of crucial importance in this case. From the comparison of reflectance curves (see Tullberg *et al.*, 2008) it

is evident that the red colour of the red form contrasts more against the background of dry *A. sylvestris* plants than the pale brown colour of the pale form does. In addition to the contrast against the background, the detectability may also be influenced by internal contrasts between differently coloured body parts. As the brightness of the pale stripes is higher than that of the red ones (A. I. Johansen *et al.*, unpublished), the internal brightness contrast between black and non-black stripes should be greater in the pale form than in the red one. It is interesting to note that this higher internal contrast did not increase detectability of the pale form. The striped pattern of pale *G. lineatum* may itself have a cryptic function matching the pale striped background of dry stems and flower stalks (pedicels) of host plants in the background; particularly when directions of the bug stripes and inflorescence striping coincide (Merilaita, 1998; Stevens *et al.*, 2006; Stobbe & Schaefer, 2008). These stripes may also create a disruptive effect of the body shape which also works for the red and black form (Merilaita, 1998; Stevens *et al.*, 2006). However, a pattern that consists only of colours present in the background, as is more the case in the pale form, have been shown to constitute a more effective camouflage than disruptive colour patterns with non-matching colour elements (Stevens *et al.*, 2006). Although we did not specifically investigate differences in background matching or disruptive contrast in our experiment, these factors could have contributed to making the pale form of *G. lineatum* harder to detect.

There was no difference in hesitation time in the first training trial between birds presented with the pale and red *G. lineatum* baits. Although colour biases have been shown for wild-caught great tits in studies using artificial prey (Ham *et al.*, 2006), the birds in our experiment were not biased against any of the forms. The birds did, however, hesitate for some time before attacking the first bait, even though it was presented with the sunflower seed visible. This hesitation for both forms could most probably be explained by the novelty of the baits, through neophobia, dietary conservatism (Smith, 1975; Marples, *et al.*, 1998) and/or an initial difficulty to recognise the baits as food items. Another explanation of the hesitation is that the birds may have had a previous negative experience with both forms of *G. lineatum* in the field and may have generalised this to the baits. If the experimental birds did have a previous negative experience of *G. lineatum*, the results of the training phase of our experiment show that great tits are highly flexible in their food choice and are able to start utilising a previously un-preferred prey once they have found it no longer unpalatable.

One possible reason for the lack of bias would be if the birds did not categorise the baits as insects as the baits were immobile, without legs and antennas, and had the seeds attached. In experiments with naïve domestic chicks (*Gallus gallus domesticus*) and wild-caught blackcaps (*Sylvia atricapilla*), birds preferred green to red insect prey, but did not show any colour preference when fruit-like prey were presented (Gamberale-Stille & Tullberg, 2001; Gamberale-Stille *et al.*, 2007). A similar non-preference for colour was found in another study with wild-caught blackcaps, using artificial food, although a preference for red was found in naïve hand-raised blackcaps (Schmidt & Schaefer, 2004).

The present experiment shows that to be pale instead of red in the late summer environment seems to be advantageous for adults of *G. lineatum*, owing to decreased probability of detection by predators. However, to sort out the major differences in selection pressures that result in the seasonal difference in defensive strategy in Swedish *G. lineatum*, may be quite complicated. There may be differences in the predator community during the year, for instance resulting in a higher risk of attack when being discovered in late summer when there are a lot of young naïve avian predators around. These naïve predators often have to learn to avoid aposematic prey, killing a lot of them in the process (Svádová *et al.*, 2009). This may favour a cryptic strategy. Also, we have observed that it takes several days for the pale adult's cuticle to harden properly after moulting, making surviving a potential attack less likely (A. I. Johansen, pers. obs.). Being in a more vulnerable state it is generally advantageous to be cryptic instead of conspicuous (Wiklund & Sillén-Tullberg, 1985). However, an initial increased vulnerability after moulting cannot be the only explanation for the more cryptic strategy in autumn, as the shieldbugs remain pale after the hardening of the cuticle. Another reason for being cryptic in autumn would be if these individuals were less chemically defended. However, previous studies indicate that both forms are aversive when attacked by birds (Tullberg *et al.*, 2008; Gamberale-Stille *et al.*, 2010).

The change to a more conspicuous red coloration in the post-hibernating adults suggests that the aposematic strategy has become more advantageous than the cryptic one. A potential selective reason for such a shift in strategy may be that the pale form cannot be cryptic on the green spring vegetation. Green colours do not occur in *Graphosoma* species (Wagner, 1956; Stichel, 1960); consequently, a potential change from one cryptic colour pattern to another may be phylogenetically constrained. An alternative reason for the change in antipredatory strategy may be that a spring camouflage would entail too great behavioural constraints on *G. lineatum*, during the reproductive period, when the bugs need to move around to find mates and places for oviposition (cf. Merilaita & Tullberg, 2005; Speed & Ruxton, 2005). In a preliminary field experiment on motility it turned out that *G. lineatum* moved a lot more in late spring than in the late summer environment (A. I. Johansen *et al.*, unpublished). Moreover, the behaviour of avian predators might play a role in selective maintenance of the red form among the post-hibernating adults. For instance, Gamberale-Stille *et al.* (2010) used live *G. lineatum* and naïve chicks (*Gallus gallus domesticus*), as predators, and found that the red form was initially more intimidating than the pale form. However, in that experiment the chicks learned to avoid both adult forms. However, the avian predators may generalise their experience asymmetrically (Gamberale-Stille & Tullberg, 1999; Svádová *et al.*, 2009) avoiding the red form of *G. lineatum* in spring more frequently than the pale one in spite of more experience with the latter from the past fall. It is also possible that the experience of birds with other red and black aposematic species (e.g. *Lygaeus equestris*, many coccinellid beetles) that emerge from hibernation earlier than *G. lineatum*, in Sweden and Central Europe, may favour the red individuals of *G. lineatum*.

There are two kinds of intertwined processes both intimately associated with the irreversible ontogenetic colour change: namely the biosynthetic pathways of the pigments and conditions allowing for their temporary or permanent arrest, and the factors regulating the onset and termination of diapause and winter dormancy. Only the integrative view taking into account such mechanisms, and the adaptive value of the resulting phenotypes will provide us with a complete picture of the coloration and its change in *G. lineatum*, an obviously complex phenomenon varying in time and space. Understanding the phylogeny and geographic variation of *Graphosoma* species is essential for any attempt to understand the evolution of their coloration.

We have provided experimental evidence that the pale form of *G. lineatum*, in Sweden, is better camouflaged in the late summer and fall than the red form, and discussed the underlying reasons for the colour change in this species. However, having discussed why it is better for *G. lineatum* in Sweden to be red after emerging from hibernation in late spring, it is apparent that some of the hypotheses suggested are still waiting to be tested.

Acknowledgements

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