

What constitutes optical warning signals of ladybirds (Coleoptera: Coccinellidae) towards bird predators: colour, pattern or general look?

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Most ladybirds (Coleoptera: Coccinellidae) possess chemical protection against predators and signal its presence by less or more conspicuous coloration, which can be considered as a warning. Most ladybirds possess a dotted pattern, although the number, shape, and size of the spots, as well as their colour, varies considerably. Almost all ladybirds have a characteristic general appearance (body shape). We considered these traits to be used in ladybird recognition by avian predators. In the present study, we compared the reactions of avian predators (*Parus major*) caught in the wild, to four differently coloured ladybird beetles (*Coccinella septempunctata*, *Exochomus quadripustulatus*, *Subcoccinella vigintiquatuorpunctata*, and *Cynegetis impunctata*) and two artificial modifications of *C. septempunctata*; the first was deprived of their elytral spotted pattern by painting it brown, the other had their elytra removed (i.e. altering their general ladybird appearance). Ladybirds with a spotted pattern were attacked less frequently than unspotted ones. Ladybirds with removed elytra were attacked much more often than any ladybird with a preserved general appearance. The results obtained in the present study suggest the high importance of the spotted pattern as well as general appearance in the ladybird recognition process. Additional experiments with naïve birds (hand-reared *P. major*) demonstrated the innateness of the aversion to two differently spotted ladybird species (*C. septempunctata* and *Scymnus frontalis*). © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, 98, 234–242.

ADDITIONAL KEYWORDS: body shape – chemical signal – prey recognition – warning coloration.

INTRODUCTION

Warning coloration has been considered as a very important antipredatory signal subsequent to early studies dealing with aposematic animals (Komárek, 2003). This signal is addressed to optically orienting predators (Edmunds, 1974). In the case of terrestrial invertebrate prey, birds are the most common visual predators (Smith, 1980; Evans & Schmidt, 1990; Schuler & Roper, 1992; Roper & Marples, 1997; Exnerová *et al.*, 2006). The most common colours used by insects to discourage predators from attacking them are bright red, orange, and yellow (Cott, 1940). These colours are distinctive so that predators quickly determine the connection between coloration and the

toxicity of the prey (Coppinger, 1969; Gittleman, Harvey & Greenwood, 1980; Harvey & Paxton, 1981; Guilford, 1986). Nonetheless, other colours, such as white, may be utilized as antipredatory signals (Lyytinen *et al.*, 1999). The importance of colour *per se* has been demonstrated several times using optically orienting predators (Sillén-Tullberg, 1985; Marples, van Veelen & Brakefield, 1994; Ham *et al.*, 2006).

Colour is not the only aspect of optical warning signals. Bright colours usually form contrasting patterns (often in combination with black or white), which should enhance the warning signal (Endler, 1978). The importance of patterns has been experimentally demonstrated using avian predators (Schuler & Hesse, 1985; Osorio, Miklosi & Gonda, 1999; Endler & Mielke, 2005). Particular parameters of colour pattern, such as symmetry and size, have

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been proposed to be of significance (Forsman & Merilaita, 1999, 2003; Forsman & Herrström, 2004; Cuthill *et al.*, 2006). Some studies revealed the possibility that even dull colours may provide a warning signal. For example, Wuster *et al.* (2004) and Niskanen & Mappes (2005) demonstrated that the brownish colour in vipers was sensed as a warning when forming a zig-zag pattern.

There are additional optical signals possessed by an insect that may be used by predators to evaluate how dangerous they are. These traits, such as body shape, body posture, and shape of legs and antennae, are often neglected in studies testing optical antipredatory signals. Nonetheless, there are several studies showing the antipredatory importance of these traits in ants (Ito *et al.*, 2004; Nelson *et al.*, 2006) and other hymenopterans (Kauppinen & Mappes, 2003).

The ladybird beetle (Coleoptera: Coccinellidae) is another example of an animal with a characteristic general appearance, and this may be important in their recognition and warning signalization. The ladybirds comprise a high portion of conspicuously coloured species (Majerus, 1994). There is amazing variability in the colour patterning of ladybirds, but the antipredatory efficiency of many of them has never been tested. However, it is believed that the colour patterning is used for signalling unpalatability to predators (Majerus, 1994). The coloration can range from a reddish or yellowish background with dark spots (*Adalia*, *Coccinella*, *Hippodamia* and many others) to black with light (red, yellow) spots (*Adalia*, *Scymnus*, *Exochomus*) or brown with light spots (*Adalia*, *Calvia*). Moreover, there are several inconspicuous species with fragmented brownish patterns (*Aphidecta*, *Rhizobius*, *Subcoccinella*, *Harmonia quadripunctata*) that can be considered as a warning, as well as cryptic, according to background circumstances (Majerus, 1994). Another trait that affects the conspicuousness of ladybirds is the type of surface on the elytra and pronotum, which can be smooth and shiny, or covered in hairs, resulting in a matt appearance. Colour variability is quite broad not only among members of the Coccinellidae, but also within particular species, where several colour forms coexist (e.g. *Adalia bipunctata*: Holloway *et al.*, 1995; *A. bipunctata* and *Adalia decempunctata*: Honěk, Martínková & Pekar, 2005, *Harmonia axyridis*: Kholin, 1990). The majority of ladybird species possess any type of spotted pattern, but the number and size of spots varies markedly (Majerus, 1994). Only a small proportion of ladybirds have plain coloration (unspotted reddish: *Coccidula rufa*; brownish: *Cynegetis*).

Despite the broad range of colours and patterns present in ladybirds, the shape of the body is very uniform within this family. Ladybirds have a quite distinct body contour, which is caused mainly by the

shape of the elytra (quite broad and convex) and broad pronotum. There are few other beetles in Europe with a similar body shape.

Ladybirds possess chemical protection against predators. In most ladybird species, noxious and often poisonous chemical substances have been found and isolated in their haemolymph; the most common comprising alkaloids, polyazamacrolides, and polyamines (Laurent, Braekman & Dalozze, 2005). Moreover, pyrazines, which are chemicals known to provide long-distance (i.e. perceived by smell, not by taste) antipredatory protection (Guilford *et al.*, 1987), were found in ladybird haemolymph (Rothschild & Moore 1987). When sensing danger, ladybirds emit poisonous and odious droplets (reflex bleeding). It has been demonstrated that chemical signals of ladybirds can prevent attacks by olfactory orienting predators such as ants (Sloggett, Wood & Majerus, 1998; Pasteels, 2007), spiders (Camarano, Gonzalez & Rossini, 2006), true bugs (HoughGoldstein, Cox & Armstrong, 1996), lacewings (Lucas, 2005), and other ladybirds (Agarwala & Dixon, 1992). The presence of toxic chemicals in ladybirds also represents a potential danger for optically orienting predators such as birds. Marples (1993) and Marples, Brakefield & Cowie (1989) demonstrated that some ladybird species are strongly toxic for birds (e.g. blue tits). Therefore, it is essential for at least some birds to recognize ladybirds precisely.

The optical signals of ladybirds vary markedly, however traits used in the recognition of dangerous prey should (i.e. based on aposematism theory) be as comprehensible as possible and thus uniform (Guilford, 1990). If the predator was to have to learn every particular form of ladybird, there would be extreme demands for predator memory and cognition (Speed, 2001). Furthermore, the presence of antipredatory chemicals is not correlated with the presence of warning coloration (Majerus, 1994). Therefore, it may be dangerous to use a particular colour combination in ladybird recognition. The general appearance (especially the shape of the body) or presence of any spotted pattern appears to be a more credible parameter for the optical recognition of ladybirds.

In the present study, we attempted to reveal the importance of particular components in the optical warning signal of ladybirds for recognition by a model bird predator. We tested the signal efficiency of several ladybird species (and their artificial modifications) using adult (caught in the wild) and hand-reared great tits (*Parus major*) as predators.

In the first experiment, we compared the response of adult tits to four ladybird species: two presumed to possess warning colour signals together with a shiny surface (*Coccinella septempunctata*: red with black spots; *Exochomus quadripustulatus*: black with red

spots), one species with fragmenting, less conspicuous coloration (*Subcoccinella vigintiquatuorpunctata*: reddish-brown background with many dark spots and matt surface) and one species with light brown spotless and matt coloration (*Cynegetis impunctata*).

In the second experiment, we compared the responses of adult tits with three varieties of *C. septempunctata*: unmodified, brown painted (warning coloration together with spotted pattern and shining surface removed) and with elytra removed (general 'ladybird' appearance altered). Additionally, we tested the innateness of the aversion to ladybirds in hand-reared great tits. We compared their response to two ladybird species possessing differently conspicuous coloration: *C. septempunctata* (red with black spots and shiny surface) and *Scymnus frontalis* (black with red spots and matt surface).

MATERIAL AND METHODS

PREDATORS

The great tit (*P. major*, Linnaeus, 1758) is an insectivorous bird, 14–15 cm long, mass 14–23 g, occurring throughout Europe and much of Asia. It is commonly used for testing the efficiency of antipredatory signals (Lindström, 1999). Its ability to recognize aposematic prey according to colour signals has been shown in several studies performed with invertebrate prey (Sillén-Tullberg, Wiklund, Järvi 1982; Lyytinen *et al.*, 1999; Exnerová *et al.*, 2003, 2006; Hagen, Leinaas & Lampe, 2003). Moreover, it has been demonstrated that great tits are able to learn the connection between unpalatability and warning signals in the red firebug (*Pyrrhocoris apterus*; Exnerová *et al.*, 2007). Furthermore, Marples *et al.* (1989) showed that some ladybird species are highly toxic to their relatives blue tits. Therefore, we may presume that recognizing ladybirds is essential for great tits, which can be considered as potential ladybird predators (Cramp & Perrins, 1993).

Adult experimental individuals were caught with mist nets in South Bohemia, Czech Republic, during the nonbreeding period in 2004–2006. Birds were kept in standard birdcages (50 × 30 × 30 cm) at lowered indoor temperature and under an outdoor photoperiod. Birds were acclimated to the laboratory conditions for 1–2 days prior to experiments. Sunflower seeds and mealworms (larvae of *Tenebrio molitor*) were provided during this acclimation. To avoid pseudo-replication, each individual was used for a single series of trials. The birds were ringed and released immediately after the experiment.

Naïve predators were obtained from nest boxes in the same forest where the adults were caught. They were taken from the boxes (four individuals from each

nest; only from broods containing at least eight chicks) at the age of 12–14 days (approximately 2 days before leaving the nest). Birds were kept in standard bird cages in groups according to kinship. A curd cheese mash (curd cheese, grated carrot, grated boiled eggs, vitamins, and crushed egg shells), commercial feeding for insectivorous birds, and mealworms were provided as diet. When birds were able to ingest prey by themselves (approximately 4 weeks after hatching), training in experimental cages started. When a bird responded to offered prey (mealworm) in the cage appropriately (i.e. attacked immediately after offering), experiments were started. After the series of experiments, birds were trained to forage (by presenting various live prey) and fly in an aviary (4 × 4 × 4 m) and, when found ready, were released.

PREY SPECIES

Five species of ladybirds were used as potential avian prey for the experiments (Fig. 1): *C. septempunctata* (Linnaeus, 1758), *E. quadripustulatus* (Linnaeus, 1758), *S. vigintiquatuorpunctata* (Linnaeus, 1758), *C. impunctata* (Linnaeus, 1767), and *S. frontalis* (Fabricius, 1787). Adult ladybirds were collected during the autumn months, and were in the stage of reproductive diapause. All ladybirds were stored at 10–15 °C for several months before the experiments. Only water in cellulose cotton was provided.

The seven spot ladybird, *C. septempunctata*, is a large species (6–8 mm long), and its coloration consists of a shiny red background of the elytra, with small black spots, and a black and white pronotum and head. It contains the alkaloids coccinelline (Tursch *et al.*, 1971) and precoccinelline (Karlsson & Losman, 1972). The pine ladybird, *E. quadripustulatus*, is medium sized (3–5 mm) and shiny black with four red spots. It contains exochomine, a dimeric alkaloid (Timmermans *et al.*, 1992). The 24-spot (or alfalfa) ladybird, *S. vigintiquatuorpunctata*, is medium sized (3–4 mm) and brownish-red, with many small dark markings. This species is somewhat variable in the number and size of spots and possesses grey hairs on the upper surface, giving the beetle a matt appearance. Its alkaloid, *N*α-quinaldyl-L-arginine-HCl, has a unique molecular structure among ladybird alkaloids (Wang *et al.*, 1996). The grass ladybird, *C. impunctata*, is medium sized (3–4 mm), pale brown, and is generally found in central Europe, usually without black markings. The upper surface is hairy with a matt appearance. Unlike the other four species, it appears cryptic rather than conspicuous. *Scymnus frontalis* is small (3 mm) and black, with four large reddish-brown spots on the elytra. The upper surface is hairy with a



Figure 1. Experimental ladybird species. A, *Cynegetis impunctata* (photo Stanislav Krejčík, meloidae.com); B, *Subcoccinella vigintiquatuorpunctata* (photo Malcolm Storey, <http://www.bioimages.org.uk>); C, *Exochomus quadripustulatus* (photo Stanislav Krejčík, <http://meloidae.com>); D, *Coccinella septempunctata* unmodified (photo Stanislav Krejčík, <http://meloidae.com>); E, *Coccinella septempunctata* with removed elytra (photo Oldřich Nedvěd); F, *Coccinella septempunctata* brown-painted (photo Oldřich Nedvěd); G, *Scymnus frontalis* (photo K. V. Makarov, <http://www.zin.ru>). Species are not depicted to scale.

et al., 2003), and causing no obstacle to motion for the beetles; (2) by excision of the elytra by scalpel; the ladybirds would then immediately extend the lower membranous wings, thus the entire ladybird appearance (shape, etc.) was altered, together with coloration and pattern (Fig. 1E). During these modifications, each ladybird was held on a piece of cellulose cotton that absorbed the released haemolymph droplets with protective compounds. At least 1 h elapsed before experimentation with the bird predator to allow volatile protective compounds to disappear from surface of the beetle, and to allow the ladybird to replenish volatiles in its haemolymph.

EXPERIMENTAL EQUIPMENT

Experiments were performed in cubic cages ($71 \times 71 \times 71$ cm). All walls except one were covered with a wire mesh (2 mm). The front wall consisted of a one-way mirror. The cage included a perch, a bowl with water, and a revolving circular feeding tray containing six cups. The bottom of each cup (diameter 5.5 cm, depth 1.5 cm) was white. Each of the cups contained a single prey item during a trial. The distance between the perch and the tray was approximately 25 cm. Illumination was generated by a daylight spectrum fluorescent tube (LUMILUX COMBI 18 W; Osram).

TRIALS

Collectively, 120 great tits were used as predators. Adult experimental birds were divided into six groups of 15 individuals. Four groups were presented with particular unmodified species of ladybirds (*C. septempunctata*, *E. quadripustulatus*, *S. vigintiquatuorpunctata*, and *C. impunctata*); two groups were presented with modified *C. septempunctata* (brown-painted and elytra-cut). Hand-reared birds were divided into two groups of 15 individuals: one group was offered *C. septempunctata* and the other *S. frontalis*.

matt appearance. The identities of defensive chemicals for the latter two species are unknown.

Two artificial non-aposematic modifications were made from the seven spot ladybirds (Fig. 1E, F): (1) by painting the upper surface with brown tempera colour, which is matt, nontoxic, non-odious (Exnerová

Two hours before the start of an experiment, a bird was placed into the experimental cage, with access to water but no food. Previous experiments showed that 2 h is sufficient to evoke food-searching but not stress. Each bird was assumed to be ready for the experiment when it attacked a mealworm in the feeding tray immediately after it was offered. Each experiment with an individual bird consisted of a series of ten trials. Control prey (mealworm) was offered alternately with experimental prey (any form of ladybird), and this was repeated five times (sequence mealworm, ladybird, mealworm, ladybird, etc.). The repetition of five successive presentations was used to reveal a possible effect of neophobia, which was previously demonstrated to be a short-term event (Marples & Kelly, 1999). The control prey was used to check the bird's motivation to feed, and the trial ended after the prey had been eaten. Each trial with experimental prey lasted 5 min. There were three possible results for a particular trial: the bird attacked and killed the offered prey; the bird attacked (handled by bill) offered prey, but the prey managed to survive; or the bird did not attack the prey. Repetition of attacks occurred only sporadically: 15 birds out of 120 attacked the prey twice (when confronted with brown painted or elytra-cut modifications of *C. septempunctata*) and two birds attacked the prey three times (with elytra-cut *C. septempunctata*). Therefore, all the results obtained were summarized for every particular bird and data were used in statistical comparisons in a binomial distribution: 1 = a certain activity was observed during at least one of the five experimental prey trials; 0 = the activity was not registered during any of the five trials with a particular bird.

STATISTICAL ANALYSIS

The binomial data were compared using generalized linear models (binomial distribution, logit link function, analysis of variance (ANOVA), and the *F*-test were used as the test criteria) with post-hoc Tukey's honestly significant difference (HSD) tests. Fisher's exact test was used in pairwise comparisons. The probability level showed clear significance without the need for Bonferroni adjustment. All tests were performed in STATISTICA, version 6 (StatSoft, Inc.).

RESULTS

KILLING OF PREY

All mealworms offered in the trials were eaten (killed). In none of the total 600 trials (120 series, each of five offers) did we record any killing of any offered ladybird; each ladybird survived 5 min of the

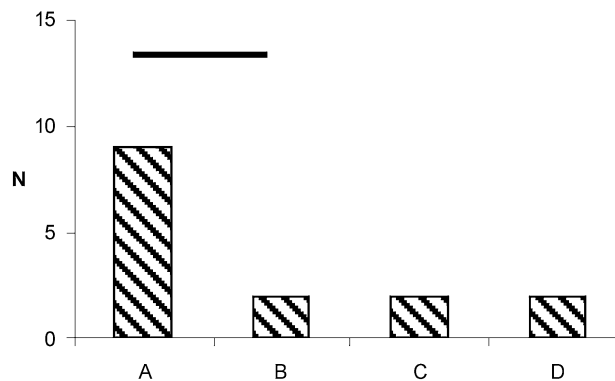


Figure 2. Numbers of great tits attacking particular ladybird species. A, *Cynegetis impunctata*; B, *Subcoccinella vigintiquatuoropunctata*; C, *Exochomus quadripustulatus*; D, *Coccinella septempunctata*. The line indicates a significant difference.

trial duration. Thus, the strongest reaction of the experimental birds comprised the attack.

COMPARISON OF FOUR LADYBIRD SPECIES

There was a significant difference in bird reaction towards the four tested ladybird species (ANOVA: $F_{60,57} = 5.197$; $P = 0.003$; Fig. 2). The number of birds attacking the brown coloured *C. impunctata* was higher (post-hoc Tukey's HSD test, $P = 0.002$) compared to those attacking the other three ladybirds.

COMPARISON OF MODIFIED COCCINELLA SEPTEMPUNCTATA

There was a significant difference in bird reaction towards the three variations of *C. septempunctata* (ANOVA: $F_{45,42} = 9.172$; $P < 0.001$; Fig. 3). The birds attacked naturally-coloured as well as brown-painted beetles less often than elytra-cut ones (post-hoc Tukey's HSD test; naturally-coloured, $P < 0.001$; brown-painted, $P = 0.014$). The reaction to naturally-coloured ladybirds did not differ from the reaction to brown-painted ones ($P = 0.096$).

COMPARISON OF ADULT AND NAÏVE PREDATORS

Both tested ladybird species (*C. septempunctata* and *S. frontalis*) were attacked by the same proportion of birds (Fig. 4). Naïve birds attacked naturally-coloured *C. septempunctata* more often than adult birds (Fisher's exact test, $P = 0.021$; Fig. 4).

DISCUSSION

KILLING THE LADYBIRDS

Although every tested ladybird species or artificial modification was at least sporadically attacked, none

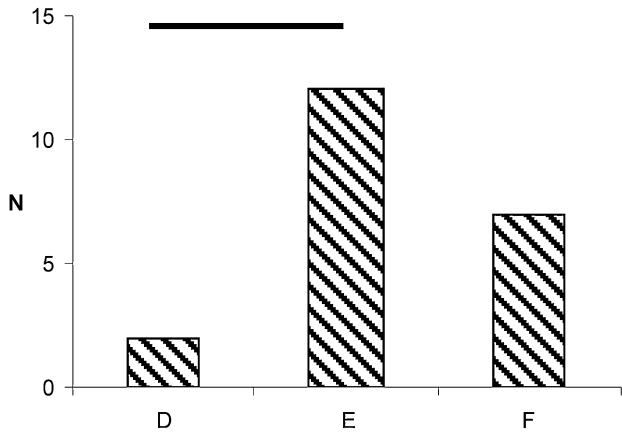


Figure 3. Numbers of great tits attacking particular modifications of *Coccinella septempunctata*. D, unmodified; E, with removed elytra; F, brown-painted. The line indicates a significant difference.

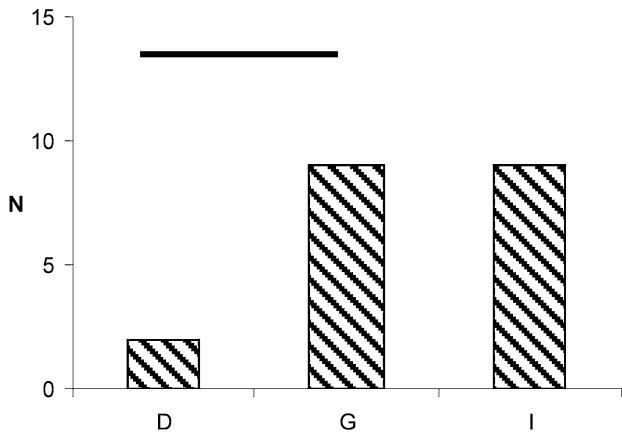


Figure 4. Numbers of adult and hand-reared great tits attacking particular ladybird prey. D, adults versus *Coccinella septempunctata*; G, hand-reared versus *Scymnus frontalis*; I, hand-reared versus *C. septempunctata*. The line indicates a significant difference.

were killed regardless of their optical warning signals. The ladybirds thus appear to be highly protected against the great tit, that should not be regarded for other warningly coloured prey. Similar experiments (Sillén-Tullberg *et al.*, 1982; Exnerová *et al.*, 2003; Veselý *et al.*, 2006) with this predator species showed that they are willing to kill and even eat several species of true bugs (Heteroptera) when their optical protection is weakened.

We assume that attacking birds were discouraged from continuing the attack by a chemical signal (i.e. sensed by smell or taste) when attacked ladybirds actively released their haemolymph, containing repelling substances (i.e. reflex blood). Thus, we assume that the chemical protection of tested ladybirds works

very well not only against insect predators (see Introduction), but also against great tit. The strong efficiency of this protection is not weakened by differences in chemical composition among tested species (i.e. containing various alkaloids and other defensive chemicals; see Material and methods).

However, the results obtained from experiments with the great tits cannot be generalized to all avian predators. Japanese quails in experiments of Marples *et al.* (1994) killed and ate mealworm beetles tainted by ladybird toxicity. Moreover, other quails fed directly with ladybirds did not show any discomfort. By contrast, strong toxicity of some species of ladybirds for blue tits was demonstrated (Marples *et al.*, 1989; Marples, 1993). We used two ladybird species in these studies. The seven spot ladybird (*C. septempunctata*) was shown to be highly toxic for young blue tits (Marples *et al.*, 1989) and the pine ladybird (*E. quadripustulatus*) caused serious discomfort and inhibited the growth of tit chicks slightly (Marples, 1993). These blue tits were offered pellets made of smashed ladybird bodies. They were willing to eat pellets containing extracts of most of the tested ladybird species, including those causing them subsequent digest problems, such that they were not repelled by the content of alkaloids (which are possibly not detectable by smell or taste). The results obtained in the present study suggest that living ladybirds possess additional protective substances causing the noxiousness. Rothschild & Moore (1987) revealed the presence of pyrazines in ladybirds' hemolymph. These chemicals were shown to provide chemical protection for several insect species (Guilford *et al.*, 1987) and are quite volatile, and thus may provide olfactory protection.

ATTACKING THE LADYBIRDS

Although no bird was found to kill any ladybird, the portion of attacking birds differed among tested ladybird forms. We may assume that this is caused by the differently effective optical protection of ladybirds. In experiments with adult birds confronted by four ladybird species, the spotless and brownish coloured *C. impunctata* was attacked more often than other species. All spotted ladybirds were protected very well; even the brownish and dark spotted *S. vigintiquatuor punctata*. If great tits would recognize particular ladybird species, we must presume that all three tested spotted species represent equal danger for them, and are more dangerous than the brown, spotless *Cynegetis* at the same time. This is in contrast with equally effective chemical protection of all four species (none was killed). Similarly, Marples (1993) and Marples *et al.* (1989) demonstrated the differing chemical impact of the seven spot ladybird

and the pine ladybird on blue tits. An alternative explanation suggests that great tits avoid all spotted species, regardless of their coloration, whereas the spotless ladybirds are put to the test of edibility. The spotted pattern appears to be the most important part of the optical warning signal, at least for the ladybird species tested in the present study. It protects even those species that have inconspicuous background colours. The significant importance of spots in the warning signalization of ladybirds is also in concordance with the variability of coloration in ladybirds. Although the colour of background as well as of spots varies considerably in ladybirds, only a small proportion does not possess any type of spotted pattern (Majerus, 1994).

The theory that *Cynegetis* is attacked more because of its absence of spots, and not because of its smaller chemical protection, is supported by experiments with brown painted seven spot ladybirds, which were attacked by approximately by the same proportion of birds as the brown coloured *Cynegetis*. By contrast to experiments conducted in several ladybird species, the brown painted form had the same chemical protection as well as other traits (e.g. body size) similar to the unmodified seven spot ladybird.

Nonetheless, even *Cynegetis* and the brown painted ladybird are not attacked by one half of birds, which suggests that they are still recognized as a ladybird. However, when the elytra of the seven spot ladybird are removed, it is attacked by more than 80% of birds (i.e. significantly more than in the case of the unmodified as well as the brown painted form). The importance of the general ladybird appearance in the antipredatory signal is thus indicated. Ladybirds possess a characteristic (oval, convex) body shape that is common among most species, regardless of size, colour or pattern (Majerus, 1994). When this shape was changed (together with loss of spots), great tits became confused and mostly attacked the ladybirds.

We can conclude that the results obtained in the present study suggest that the general appearance and spotted pattern suffice to explain ladybird recognition, at least by some birds. This could mean that ladybirds may not be forced by strong selective pressure to unified coloration, as are many other groups of warningly coloured animals (Guilford, 1990). This relative selective freedom may explain the wide palette of colours present among ladybirds (Majerus, 1994). The theory of importance of general body shape in the antipredatory signal of ladybirds is also supported by the high colour uniformity (i.e. red background with seven melanization centres on each elytron) of members of the subtribe *Hipodamiina*, which have a completely different appearance (i.e. elongated and depressed body).

The importance of optical signals unrelated to coloration in antipredatory signalization has often been neglected. Chemical signals and colour pattern are always considered to be the most important cues in aposematic prey recognition (Guilford, 1990). However, the warning signal should be clear and comprehensible (Guilford, 1988); a uniform and distinct general appearance may comply with this. Two studies have considered the importance of optical signals unrelated to coloration in antipredatory optical signalization and, interestingly, both dealt with non-avian predators. Nelson *et al.* (2006) demonstrated mantises to react to ants similarly to their reaction to ant-mimicking jumping spiders (i.e. equal in body shape, not in chemical signals). Kauppinen & Mappes (2003) showed the particular importance of the characteristic shape of wasp bodies in optically repelling dragonflies. Artificially nonstriped wasps were attacked less often than identically coloured flies. These results suggest that other optical traits may play a significant role in the recognition of dangerous animals.

The results obtained in the present study with respect to ladybirds cannot be generalized to other optically signalling insects. The role of precise coloration pattern is rather different in coreoid true bugs, which comprise another well described and studied group of aposematic insects. Experiments on red firebugs and great tits (Exnerová *et al.*, 2003) revealed that, in contrast to natural ones, the brown-painted firebugs were not only attacked, but also frequently killed. Even mutants of bugs with native black patterning, but differing in background colour, were less protected than naturally-coloured individuals. Sillén-Tullberg *et al.*, (1982) demonstrated a higher attack rate to seed bug (*Lygaeus equestris*) larvae when their background colour was grey instead of red. Similarly, Exnerová *et al.* (2006) showed a lower protective function of yellow and white backgrounds instead of red, in the firebug (*P. apterus*). By contrast to ladybirds, the general appearance (as well as black pattern) has no importance in coreoid true bug recognition by great tits, or, from the point of view of cognitive science, great tits do not possess a general concept (Shettleworth, 1998) of coreoid true bugs.

In experiments with naïve, hand-reared great tits, the partial innateness of aversion to ladybirds was demonstrated. Comparable experiments (Exnerová *et al.*, 2007), using red firebugs (*P. apterus*) as prey and naïve great tits as predators, showed practically no innate wariness. It is interesting that the firebug possesses the black-red colour pattern as in many ladybird species. Although at least some ladybirds are strongly toxic to some tits (Marples *et al.*, 1989), eating the firebug may only cause slight discomfort, such that the penalty is not as high. Therefore, selec-

tion pressure for innate aversion to the ladybird is stronger than that to the firebug. This type of explanation could also be used to clarify the different role of general appearance in the recognition of ladybirds and coreoid true bugs. However, we have insufficient knowledge of the general danger posed by both of these insect groups.

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