Different reactions to aposematic prey in 2 geographically distant populations of great tits

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Variation in predator behavior toward aposematic prey was frequently studied at interspecific and individual levels, but interpopulation differences have been neglected. Geographic differences in prey fauna offer an opportunity to test their implications for predator behavior. It can be expected that 1) predator populations inhabiting environments with high diversity of aposematic prey are more neophobic than those living in areas where aposematic prey are scarce, and 2) different levels of neophobia jointly with avoidance learning affect selection on aposematic prey. We compared the behavior of wild-caught great tits (Parus major) from Bohemia and Central Finland toward aposematic firebugs (Pyrrhocoris apterus), nonaposematic firebugs, novel objects and novel palatable nonaposematic prey. Finnish and Bohemian birds did not differ in their novel-object exploration, but Finnish birds hesitated longer than Bohemian birds before resuming feeding next to a novel object. Latencies to attack novel palatable prey did not differ and were not correlated with the attitude toward novel objects. Tits from the Bohemian population mostly avoided aposematic firebugs and attacked nonaposematic ones. Finnish birds were more likely to attack both firebug color forms, and their attack latencies were correlated with latencies of attacking novel palatable prey. Thus, Bohemian birds avoided the aposematic prey, but were not more neophobic than Finnish birds. These results suggest that differences between Finnish and Bohemian birds in behavior to aposematic prey do not follow differences in exploration strategy and neophobia. The observed differences can be explained by a different experience with local aposematic prey communities.

Key words: aposematism, exploration, geographic differences, neophobia, Parus major, Pyrrhocoris apterus.

INTRODUCTION

Avoidance of aposematic prey usually involves several cognitive mechanisms that affect the behavior of predators (Ruxton et al. 2004). Reaction toward prey may be influenced by neophobia (e.g., Copping 1969, 1970; Exnerová et al. 2010) or dietary conservatism (Marples et al. 1998; Marples and Kelly 1999), and by inherited (Smith 1975; Lindström et al. 1999a) or learned (e.g., Lindström et al. 1999b; Exnerová et al. 2007; Aronsson and Gamberale-Stille 2008; Barnett et al. 2012) aversions against certain warning signals and their combinations (Marples and Roper 1996; Rove and Guilford 1996; Lindström et al. 2001). The response of a predator to warning signals is affected by associative learning, the degree of which is influenced by memorability, prey recognition, discrimination, and generalization (Roper and Redston 1987; Gamberale-Stille and Tullberg 1999; Speed 2000; Ham et al. 2006; Svádová et al. 2009). Given the complexity of the cognitive processes, which contribute to the formation of the avoidance of aposematic prey, it is not surprising that there exists a considerable variation in behavior of different predators toward a defended prey species (Brower 1988; Exnerová et al. 2003; Endler and Mappes 2004; Valkonen et al. 2012; Nokelainen et al. 2014).

Predators from different taxa may react differently to a particular prey species, and several mechanisms have been discussed as potential factors responsible for the variation: the energetic requirements of a predator (i.e., body size or hunger level) are important in determining whether or not the predator decides to attack and consume a defended prey (Exnerová et al. 2003; Barnett et al. 2007; Halpin et al. 2014). Also, sensory and cognitive abilities of predators can be highly variable (Hart 2001; Sol et al. 2005) influencing...
their ability to learn to avoid aposematic prey (Endler and Mappes 2004). Closely related species also frequently differ in their reactions to novel environments, objects and food; the neophobia level may be correlated with the degree of habitat and foraging specialization (Greenberg 1989; Mettke-Hofmann et al. 2009, 2012; Tebbich et al. 2009).

Variation in reactions to aposematic prey also exists among conspecific predators, where the initial wariness and rate of avoidance learning may be correlated with personality traits (Exnerová et al. 2010). On the other hand, the rate of incorporation of a novel food item into an individual’s diet represents another process: dietary conservatism (Marples et al. 1998; Marples and Kelly 1999), which is independent of personality and not correlated with neophobia (Marples and Mappes 2011). The behavior of predators toward aposematic prey may also be correlated with their age (Lindström et al. 1999a; Exnerová et al. 2006; Langham 2006; Mappes et al. 2014), and due to the importance of learning also highly affected by individual experience (Exnerová et al. 2007; Ihájainen et al. 2008; Barnett et al. 2012; Hotová Svádová et al. 2013).

In contrast to interspecific and individual differences in behavior toward aposematic prey, the potential differences between conspecific populations, namely those living in geographically distant areas and different habitats, have not been studied. Despite being interesting per se, the knowledge of potential geographical differences may be important for the generalization of results based on studies of different populations of a particular species of predator. Individuals from geographically distant populations may react differently to aposematic prey simply because of different individual experience with local aposematic prey. Alternatively, the behavior toward aposematic prey may reflect population-specific differences in neophobia (see Liebl and Martin 2014) and exploration strategies evolved for living in different conditions such as prey diversity and frequency of noxious prey. Individuals from populations living in different conditions (e.g., different predation pressure and environment stability) differ in their exploration of a novel environment, and their reactions to novel objects and novel food (Martin and Fitzgerald 2003; Brydges et al. 2006; Echeverría and Vassallo 2008; Korsten et al. 2010; Liebl and Martin 2014). Likewise, individuals from migratory populations may be more neophobic than their resident conspecifics (Mettke-Hofmann et al. 2013).

In this study, we investigated geographical differences in response to novel stimuli and reaction to aposematic prey in the great tit (Parus major L., 1758), a small passerine which is mainly insectivorous during spring and summer, although in autumn and winter, when insect prey become scarcer, adds berries and seeds to its diet (Cramp and Perrins 1993). The great tit is a resident species inhabiting a wide range of woodland habitats in the Palearctic region, and its distribution covers the whole Europe including the far North (Cramp and Perrins 1993). In recent years, the great tit has become a model species in studies on aposematism and mimicry. Because such studies are based on experiments with birds from various localities across Europe (e.g., Sillén-Tullberg 1985; Lindström et al. 1999a, 1999b; Exnerová et al. 2006), it is worth testing whether birds from different populations behave in the same way. As a model aposematic prey we used the firebug (Pyrrhocoris apterus), which is conspicuously red-and-black colored and unpalatable for small passerine birds (Exnerová et al. 2003). The firebug is widespread in the Palearctic, but it is absent in Britain and most of Northern Europe.

By comparing the behavior of wild-caught great tits from 2 geographically distant areas, Bohemia and Central Finland, we tested the following hypotheses concerning the reaction of these birds toward aposematic prey: 1) Differences in behavior toward aposematic firebugs follow differences between the 2 populations in their behavior toward novel palatable prey and other types of novel objects, that is, they reflect the levels of individual exploration and neophobia. 2) The 2 populations exhibit specific differences concerning the aposematic firebugs. The Finnish birds are expected to be more willing to attack aposematic firebugs because of their lack of experience with this type of the aposematic prey in their natural environment. 3) Birds from both populations avoid attacking firebugs regardless of their experience. This may happen if the avoidance of aposematic prey has a strong genetic basis or the avoidance learning of prey with a given warning signal is generalized to other prey whose signal is similar enough. Because the local diversity of aposematic prey is important for the interpretation of the behavior of birds, we also analyzed data on the distribution of aposematic and nonaposematic species of Heteroptera between the 2 compared areas.

### MATERIALS AND METHODS

Experiments were carried out at Konnevesi Research Station, University of Jyväskylä (Central Finland) and in Prague at the Faculty of Science, Charles University (Bohemia) during autumn 2012. In order to standardize the phenological differences between the 2 localities, the experiments were conducted during October in Central Finland and during November in Bohemia.

### Comparison of occurrence of aposematic species of Heteroptera in Central Europe and Central Finland

In order to compare the composition of the fauna of true bugs from both areas, we gathered data on the occurrence of heteropterans, particularly of the aposematic species, in Central Europe and Central Finland. Data for Central Europe were obtained combining the whole heteropteran faunas of Germany (species included in Wachmann et al. 2004, 2006, 2007, 2008) and Czech Republic (Štys P, unpublished data). As for Central Finland, we covered species occurring in Finland at the latitude of Konnevesi (62°38′N), or likely to occur there (known to occur slightly more to the South or to the North), as shown by distributional maps (Rintala and Rinne 2010). Purely coastal species were excluded. Taxonomically, we considered only the terrestrial Heteroptera (Leptopodomorpha, Cimicomorpha, and Pentatomomorpha s. lat.). In their adult stage, the dorum of those species regarded as aposematic is uniformly colored with bright white, yellow, orange or red, or with a combination of any of those and a contrasting dark pattern. For the purpose of this analysis, we also classified as aposematic those species having nonaposematic morphs as well. The dull whitish, yellowish or reddish taxa/morphs were not taken as aposematic (e.g., some Miridae: Phylinae). All terrestrial true bugs are known to have a chemical defense (Schuh and Slater 1995).

### Predators

Altogether we tested 100 wild-caught great tits, 50 from each of 2 geographically distant populations: 1) Konnevesi in Central Finland (62°38′N, 26°19′E) and 2) Prague in Bohemia (Czech Republic, 50°04′N, 14°26′E). The words “Finnish” and “Finland,” whenever used without qualification in the text, always refer to Central Finland at about Konnevesi latitude. The sex and age of both experimental groups were balanced (Central Finland: 30 males and
20 females, 19 yearlings and 31 adults; Bohemia: 29 males and 21 females, 17 yearlings and 33 adults). The birds from both populations had similar body weight (Bohemian birds—mean 16.5 g ± 0.9 g, N = 50; Finnish birds—mean 17.1 g ± 1.0 g, N = 50).

The habitats around Konnevesi include mainly sparse mixed forest with a low underground of moss, grasses and sedges, herbaceous plants and shrubs; the forest is interspersed with large clearings including also buildings and human settlements; a brook and meadows along lake sides are present as well. The Prague locality consists mainly of large city parks with coniferous and broad-leaved trees; the parks are surrounded by roads and residential houses with small patches of ruderal vegetation interspersed. The major park is an old botanical garden, which represents various Bohemian habitats, and also includes several pools and a small brook. The birds were trapped in the autumn, when overwintering birds typically move around (Čepák et al. 2009). Thus, birds in each locality were likely to be coming from surrounding areas as well.

Birds were caught using food-baited traps (Central Finland, see Ham et al. 2006 for details) or mist nets placed near the feeders (Bohemia) during autumn 2012. They were housed individually in cages (50 cm × 40 cm × 50 cm in Prague; 65 cm × 50 cm × 80 cm in Konnevesi) under natural light conditions and were kept on a diet consisting of mealworms (larvae of Tenebrio molitor L., 1758), peanuts, sunflower seeds and water ad libitum. The birds were allowed to habituate to the laboratory conditions for 5–7 days before the experiment. Each bird was used only once in each experiment. After the experiment they were ringed individually and released in the locality of their capture.

Prey

As aposematic prey we used brachypterous adult firebugs [Pyrrhocoris apterus (L., 1758); Heteroptera], which possess a conspicuous red-and-black coloration. The species’ defensive secretion from metathoracic glands containing mainly aldehydes (Farine et al. 1992) makes this insect distasteful for small passerine birds including great tits (Exnerová et al. 2003). The firebugs live on the ground and partly also on trees, and feed mainly on seeds of Malvaceae (herbaceous species and linchen tree, Tilia) and locust tree, Robinia pseudacacia (Kristenová et al. 2011; Hotová Svádová et al. 2014). They are widespread in the Palearctic but absent in most of Northern Europe; their range does not exceed the latitude of 60°N (Aukema and Rieger 2001; Rintala and Rinne 2010).

A nonaposematic variant of the firebugs lacking the red-and-black color pattern was obtained by painting their upper parts with dark brown watercolor dye and chalk. We used these color-manipulated bugs to test the specificity of birds’ reaction toward the firebugs’ warning coloration, as we needed prey that did not differ from the aposematic prey in any other trait (size, body shape, composition of defensive secretion, and so forth). The dye used to modify the visual part of the firebug warning signal was odorless and nontoxic, and the chemical defense of these artificially made nonaposematic firebugs was unchanged (see Exnerová et al. 2003).

The firebugs were collected in Prague (Czech Republic). They were kept at a temperature of 24 ± 1 °C and a light: dark cycle of 16:8 h, reared on linchen seeds (Tilia cordata) and provided with water ad libitum.

Mealworms (larvae of Tenebrio molitor) were used as a palatable control prey to check the foraging motivation of birds before starting a trial with experimental prey. We used nymphs of Jamaican field crickets [Grillus assimilis (Fabricius, 1775)] carrying a bright-blue paper sticker attached to their dorsal side as a novel, edible, nonaposematic prey to test the level of birds’ food-specific neophobia. The size of crickets offered in experiments matched the average size of tested firebugs (i.e., 10–12 mm). The sticker covered most of the cricket’s dorsum, leaving its antennae and legs visible.

In a preliminary experiment (involving 2 other groups of 20 wild-caught birds, both from Bohemia), great tits hesitated longer before attacking crickets with a blue sticker (mean 179.2 s ± 28.2, N = 20) than before attacking those without the sticker (mean 40.9 s ± 12.4, N = 20; Mann-Whitney U-test: Z = −3.92, N = 40, P < 0.001), which they attacked with similar latencies as familiar mealworms (mean 24.6 s ± 10.5; Wilcoxon matched pairs test: Z = 1.49, N = 20, P = 0.135). These results indicate that crickets with the blue sticker represent a stimulus sufficiently novel to increase attack latency.

Experimental design and equipment

Experiments were designed to compare the exploration behavior, levels of neophobia, and specific reactions to aposematic and novel palatable prey between 2 populations of great tits. Each bird was tested individually in 4 separate tests in the following order, which was identical for all the birds: 1) exploration test with a novel object, 2) neophobia test with a novel object placed near the food bowl, 3) test of reaction toward novel palatable prey, and 4) test of reaction toward aposematic firebug or its nonaposematic brown-painted variant. This way the recent aversive experience with firebugs did not affect the reactions of birds toward novel objects and palatable prey, and the order per se did not influence the comparison between populations.

Exploration and neophobia tests

To study exploration behavior and neophobia level we carried out 2 types of novel-object tests: 1) a novel object presented in a neutral location, and 2) a novel object attached to the food bowl. A novel object presented in a neutral location is frequently used to measure exploration behavior and neophobia, because the bird is not forced to approach the novel object, and when it does, it indicates its interest in exploring the object (e.g., Verbeek et al. 1994; Mettke-Hofmann et al. 2002; Drent et al. 2003). The presentation of a novel object close to the food bowl is regarded as a measure of neophobia, because it creates a conflict between foraging motivation and motivation to avoid a novel object; the bird has to overcome the neophobia to come close to the object and feed (Mettke-Hofmann et al. 2002; Feenders et al. 2011; Mettke-Hofmann 2012). Although exploration and neophobia may be correlated as they represent personality traits (van Oers et al. 2004), they are considered to be 2 distinct responses to novel stimuli (Greenberg and Mettke-Hofmann 2001).

In the exploration test, we used a bright-blue pen attached to one of the perches close to the front wall of the home cage. We measured the latency to peck at the novel object. The test lasted 10 min (maximum) and was terminated earlier if the bird pecked at the novel object. Food and water were freely available at all times.

In the neophobia test, we used a pink plastic clothes-peg attached to the food bowl placed on the home-cage floor. The birds were deprived of food for 1 h before the test to increase their foraging motivation. We recorded the latency to feed near the novel object. The test lasted 10 min and was terminated earlier if the bird started to feed in presence of the novel object. To control for potential differences in foraging motivation, we also carried out a control test under the same conditions but with the peg absent.
Novel and aposematic prey tests

Tests with novel palatable prey and firebugs were performed in experimental cages and followed one after another. Experimental cages used in Bohemia and in Central Finland were of similar size (70 cm × 70 cm × 70 cm in Bohemia and 50 cm × 50 cm × 70 cm in Central Finland); they were made of plywood and a wire mesh, and equipped with a perch and a water bowl. The front wall of cages used in Bohemia was made of 1-way glass; the birds in Central Finland were observed through a small mesh-covered window in the cage wall. Illumination of the cages simulated the natural daylight spectrum (including UV wavelengths). The tested prey was put into the cage in a glass Petri dish placed on a sliding food tray on the cage floor. Despite of difference in the cage sizes, the distance between the food tray and the closest perch was identical (35 cm). All the prey types appeared conspicuous on the light beige background of the plywood food tray. Prior to the experiments, the birds were habituated to the experimental cages, and they were deprived of food for 2 h to increase their foraging motivation. The experiments were video-recorded and the birds’ behavior was continuously registered using Observer XT 8.0 software.

Novel palatable prey test

The novel, palatable, nonaposematic prey was represented by a Jamaican field cricket with a blue-colored paper sticker attached to its dorsal side. The experiment consisted of a sequence of 5-min (maximum duration) trials. At the beginning of the sequence, we offered the bird a mealworm as a control prey to check its foraging motivation. When the bird consumed a mealworm, it was offered a cricket in the subsequent trial. If the bird did not attack the cricket within the time limit, the sequence continued with another mealworm trial followed by another cricket trial up to a maximum of 3 cricket presentations. We measured the latency to attack (touch, peck, or seize) the cricket and recorded whether the cricket was killed and eaten.

Firebug test

The birds from both Bohemian and Finnish populations were subdivided into 2 experimental groups of 25 birds each, with similar proportion of yearlings and adults, and males and females. One group was tested with aposematic red-and-black firebugs, and the other one with manipulated (brown-painted), nonaposematic (but still unpalatable) firebugs.

The test consisted of a sequence of alternating trials in which the birds were presented either with a control prey (mealworms) as a check of foraging motivation or with a firebug. Each trial lasted 5 min at most, and was terminated earlier if the bird attacked the prey. The sequence always started with a mealworm trial. When the bird consumed a mealworm, it was offered a firebug in the subsequent trial. If the bird did not attack the firebug within the time limit, the sequence continued up to a maximum of 20 firebug presentations. We measured the latency to approach and attack (to touch, peck, or seize) the firebug and recorded whether it was killed, thrown away, or eaten. If the bird attacked one of the firebugs, we kept offering them (alternating with mealworms) until the bird left untouched 3 firebugs in a row, which was considered an avoidance-learning criterion.

Data analysis

A Cox hazard regression was used to analyze the differences between Bohemian and Finnish great tits in exploration behavior toward a novel object (the latency to peck at the blue pen) and in neophobia (the latency to feed in presence of a novel object, the pink clothes-peg). Localities of bird origin, and bird’s sex and age were used as explanatory variables. In the analysis of neophobia, we included the control (peg absent) latencies in the model as a covariate. The model selection procedure started from the model including all possible 2-way interactions of locality, age and sex, and was subsequently simplified. Model selection was conducted in a hierarchical manner based on the significance of the terms in the model.

A Cox hazard regression was also used to analyze the behavior of birds to novel palatable prey (cricket with blue sticker), aposematic or nonaposematic firebugs, and control familiar prey (mealworm offered at the beginning of the experiment). As a response variable we used latency to attack each prey type (cricket, firebug, or mealworm). Locality, bird’s sex and age, and in case of firebugs also their coloration (aposematic, nonaposematic), were used as explanatory variables. The model selection procedure was similar to that used for analyzing reaction to a novel object.

We computed Spearman rank correlations between latency to peck at the novel object in the exploration test, latency to feed in the presence of novel object in the neophobia test, and latencies to attack the novel palatable prey and the firebugs. To check whether the birds considered crickets with blue stickers a novel prey, we compared attack latencies between crickets and mealworms offered in the trial preceding the cricket test; the latencies were compared by Wilcoxon matched pairs test.

We used the number of attacked firebugs before a bird stopped attacking them as an indicator of learning. A generalized linear model with Poisson distribution was used to analyze the data. The model selection was based on significance of the terms in the model. Bird sex and age, as well as locality of bird origin and firebug coloration, and all possible 2-way interaction terms were used as explanatory variables. All analyses were conducted using R 2.11.1 and the lme4 package.

RESULTS

Comparison of occurrence of aposematic species of Heteroptera in Central Europe and Central Finland

Fauna of terrestrial Heteroptera of Central Europe comprises 922 species, 766 (83%) of which are nonaposematic, and 156 (17%) aposematic; in Central Finland 262 (90%) out of 292 species of Heteroptera are nonaposematic, whereas 30 (10%) are aposematic. Thus, the less speciose fauna of Central Finland (32% species as compared with Central Europe) has not only a smaller absolute number of aposematic species (19% as compared with Central Europe) but also the proportion of aposematic species in the fauna is 0.59 times smaller than it should be in accordance with a uniform decrease in species diversity with increasing latitude (chi square = 7.75, df = 1, and P = 0.006).

Exploration and neophobia

We fitted Cox hazard regression model to explain the variation in exploration of a novel object (blue pen). However, no significant differences between the Bohemian and Finnish birds were detected (Table 1, Figure 1). Most birds approached the object and pecked at it within the time limit. The average pecking latency was 325 ± 21.5 s (N = 100).
Our final Cox hazard regression model explaining the birds’ latency to feed in proximity of a novel object (pink peg) included locality, bird age, and the interaction of both (Figure 1, Table 2). In general, Finnish birds hesitated longer compared with those from Bohemia. Among all the birds, Bohemian adults were the least neophobic, which caused the effect of the interaction between locality and age of birds (Figure 1, Table 2).

Latencies measured in exploration and neophobia tests were significantly correlated in birds from both populations (Finnish birds: \( r_s = 0.60, t = 5.15, df = 48, P < 0.05 \); Bohemian birds: \( r_s = 0.29, t = 2.10, df = 48, P < 0.05 \)).

Reaction to novel palatable prey

Most birds attacked the novel palatable prey (blue cricket) in the first or second trial (mean \( 357 \pm 37.2 \) s, \( N = 100 \)). The birds from both localities hesitated longer before attacking the blue crickets than before attacking familiar mealworms (Wilcoxon matched pairs test: Finnish birds, \( Z = 5.56, N = 50, P < 0.01 \); Bohemian birds, \( Z = 5.23, N = 50, P < 0.01 \)).

Table 1

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When an interaction is indicated (*), the main effect of the term is also included in the model. Degrees of freedom and significances are given for the excluded term in the model.

Table 2

<table>
<thead>
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<td>−0.103</td>
<td>0.918</td>
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<td>Sex (males)</td>
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<td>0.998</td>
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<td>Locality (Bohemia): age (juv.)</td>
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<td>0.519</td>
<td>−1.827</td>
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Interaction terms of the sex and age, and the locality and sex were removed from the model because they were not significant (\( z < \pm 1, P > 0.36 \)).

Figure 1

Latency of Bohemian and Central Finnish great tits to peck at a novel object (blue pen; exploration test; open bars) and their latency to start feeding after a novel object was attached to the food bowl (pink peg; neophobia test; gray bars).

Reaction to aposematic and nonaposematic firebugs

A similar proportion of Finnish and Bohemian birds attacked nonaposematic firebugs (chi square = 1.75, df = 1, and \( P = 0.185 \)). Aposematic firebugs were mostly attacked by Finnish birds, and mostly avoided by Bohemian birds (chi square = 8.33, df = 1, and \( P = 0.004 \)).

No significant effect of locality of bird origin was found explaining the variation in attack latencies (Figure 2, Table 3). However, nonsignificant trend for adult birds to be less hesitant than yearlings was detected (Table 3). We did not find a significant effect explaining the variation in latency to attack the mealworm offered just before the novel-prey test either (all \( P \) values of main effects (locality, age, sex) and their interactions were > 0.230); this indicates that all the birds entered the test with similar foraging motivation.

Attack latencies did not correlate with the latencies measured in exploration (Finnish birds: \( r_s = 0.04, t = 0.27, df = 48, \) NS; Bohemian birds: \( r_s = 0.02, t = 0.12, df = 48, \) NS) and neophobia (Finnish birds: \( r_s = 0.06, t = 0.40, df = 48, \) NS; Bohemian birds: \( r_s = 0.16, t = 1.10, df = 48, \) NS) tests.

Table 3

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Interaction terms of the sex and age, and the locality and sex were removed from the model because they were not significant (\( z < \pm 1, P > 0.36 \)).

Figure 2

Latency of Bohemian and Central Finnish great tits to attack a novel prey (cricket with a blue sticker; open bars), nonaposematic firebug (brown-painted; light gray bars), and aposematic red-and-black firebug (wild-type; dark gray bars).
Cox hazard regression model explaining the variation in latencies to attack the firebugs revealed a significant interaction between firebug coloration and locality of bird origin (chi square = 3.95, df = 1, P = 0.047). Other terms included in the model were: locality, age and sex of the bird, and firebug coloration. Bohemian birds were more hesitant to attack aposematic firebugs than Finnish birds, but there was no difference in latencies to attack nonaposematic firebugs (Figure 2).

In Finnish birds, the latencies to attack both aposematic and nonaposematic firebugs correlated with the latencies to attack novel palatable prey (aposematic bugs: $t = 0.46$, $t = 2.46$, df = 23, and $P = 0.047$). Other terms included in the model were: locality, age and sex of the bird, and firebug coloration. Bohemian birds were more hesitant to attack aposematic firebugs than Finnish birds, but there was no difference in latencies to attack nonaposematic firebugs (Figure 2).

### Table 3

<table>
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<td>0.9049</td>
</tr>
<tr>
<td>Sex * age + sex * locality</td>
<td>Sex * locality</td>
<td>1</td>
<td>0.3953</td>
</tr>
<tr>
<td>Age + sex + locality + sex * age</td>
<td>Locality</td>
<td>1</td>
<td>0.9588</td>
</tr>
<tr>
<td>Age + sex + sex * age</td>
<td>Sex * age</td>
<td>1</td>
<td>0.2273</td>
</tr>
<tr>
<td>Age + sex</td>
<td>Sex</td>
<td>1</td>
<td>0.3675</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>1</td>
<td>0.1283</td>
</tr>
</tbody>
</table>

When an interaction is indicated (*), the main effect of the term is also included in the model. Degrees of freedom and significances are given for the excluded term in the model.

### Table 4

Model fitting of Poisson GLM explaining the number of firebugs (either aposematic or nonaposematic) attacked by Bohemian and Central Finnish great tits

<table>
<thead>
<tr>
<th>Model</th>
<th>Term removed</th>
<th>df</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locality * sex + locality * age + locality * color + age * color + sex * color</td>
<td>Age * color</td>
<td>1</td>
<td>0.9811</td>
</tr>
<tr>
<td>Locality * sex + locality * age + age * color + sex * color</td>
<td>Locality * age</td>
<td>1</td>
<td>0.8621</td>
</tr>
<tr>
<td>Locality * sex + age * color + sex * color</td>
<td>Locality * sex</td>
<td>1</td>
<td>0.8452</td>
</tr>
<tr>
<td>Age + locality + sex + color + locality</td>
<td>Age</td>
<td>1</td>
<td>0.5427</td>
</tr>
<tr>
<td>color + sex * color</td>
<td>None</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If interaction is indicated (*), the main effect of the term is also included in the model. Degrees of freedom and significances are given for the excluded term in the model. Our final model (see details in Table 5) is highlighted in bold.

### DISCUSSION

Environmental factors, particularly food availability and predators, are suggested to be the main drivers selecting for differences in foraging behavior among local bird populations (e.g., Shochat et al. 2004). However, we still have very limited understanding of how such differences arise, and whether certain behavioral traits are selected together or independently. We compared several foraging-related traits in 2 geographically distant populations of great tits. Those populations have presumably experienced different selective environments in terms of habitats, diversity and abundance of noxious prey. Although our experiments are not able to discern whether the observed differences already reflect local adaptations or whether they reflect differences in experience, our results raise some interesting points regarding the nature of differences in the reaction of wild-caught birds to aposematic prey and how they are correlated with exploration behavior and neophobia.

### Exploration and neophobia—comparison of Finnish and Bohemian birds

Finnish and Bohemian great tits did not differ in their tendency to explore a novel object placed in a neutral location in their home.
cage. However, Finnish birds appeared more neophobic, and hesitated longer than Bohemian birds before starting to feed next to a novel object attached to their food bowl. Our results suggest that environmental conditions both in Bohemia and Central Finland may, in spite of their differences, favor similar exploration tendencies in great tit populations. Similarly, Miranda et al. (2013) did not find any differences in novel object exploration between 2 populations of European blackbirds (Turdus merula) living in different habitats.

The extent of object neophobia has been frequently found to differ between conspecific populations (e.g., Martin and Fitzgerald 2005; Mettke-Hofmann et al. 2013; Miranda et al. 2013, but see Echeverría and Vassallo 2008; Bókony et al. 2012). Several factors may explain the greater object neophobia of Finnish great tits in our experiment. First, Finnish birds may be more cautious to approach novel objects near their feeding place due to greater vigilance caused by higher frequency of potential predators as can be expected in a less populated and more natural landscape. In sticklebacks (Gasterosteus aculeatus), individuals living in areas with greater predation pressure are less bold and more neophobic than those inhabiting areas with less predators (Bell 2005; Dingemanse et al. 2007; Brydges et al. 2008). Second, Finnish birds may exhibit greater object neophobia because of a larger proportion of migrating individuals (Čepák et al. 2008). Although the great tit is mostly a resident species, some individuals (mostly yearlings) undergo short-distance autumnal migration (Cramp and Perrins 1993). Greater neophobia of birds from migratory populations than from the resident ones has been found in 2 New World blackbird species (red-winged blackbird Agelaius phoeniceus and Brewer’s blackbird Euphagus cyanocephalus); the difference may have been caused by residents having higher costs of missing new opportunities in the seasonally changing environment (Mettke-Hofmann et al. 2013). Lesser neophobia of Bohemian birds may be also partly explained by their more urbanized locality. Birds from urban populations may be bolder and less neophobic than their rural conspecifics, as is the case in European blackbirds (Turdus merula; Miranda et al. 2013), and song sparrows (Melospiza melodia; Evans et al. 2010).

Behavior of Finnish and Bohemian birds toward novel palatable prey

Finnish and Bohemian great tits did not differ in their behavior toward a novel palatable prey, the cricket with a blue sticker attached. They attacked the crickets with similar latencies, and mostly killed and consumed them. This suggests that the hesitation behavior toward novel prey does not reflect any general difference in local prey communities and frequency of potentially dangerous prey.

Surprisingly, we did not find any correlation between the behavior of birds toward novel prey and their reaction to novel objects (exploration and neophobia) in the 2 populations studied. In juvenile Dutch great tits coming from lines selected for opposed personality traits (Drent et al. 2003), the generally more neophobic “slow explorers” hesitated longer before attacking novel prey (red-and-black firebugs) than the less neophobic “fast explorers” (Exnerová et al. 2010). There are several mutually nonexclusive factors possibly responsible for the difference between the results of our present and previous studies (Exnerová et al. 2010): 1) The inherited correlation between food neophobia and object neophobia may be prominent in naïve juvenile birds. In contrast, adult wild-caught individuals may not show such a correlation due to their experience with various types of both palatable and unpalatable prey, as well as experience with food shortage periods. In wild-caught tits living outside the breeding season in small flocks, the food neophobia may also be influenced by the position of an individual in the flock hierarchy (Farine et al. 2015). In black-capped chickadees (Poecile atricapillus), for instance, subordinate individuals are less neophobic than dominant ones (Am et al. 2011). 2) The correlation may be more apparent in birds coming from the lines selected for the opposed personalities (Drent et al. 2003), than in birds from natural populations with possibly less extreme values of personality traits. 3) Food neophobia may be correlated with other personality traits in some populations (Dutch; Exnerová et al. 2010), but not in others (Finnish and Bohemian, this study). Bókony et al. (2012) found that food neophobia in house sparrows (Passer domesticus) correlated with activity, risk-taking, and object neophobia only in 1 of 4 Hungarian populations studied. In Kenya none of the 0 populations of house sparrows tested showed any relationship between exploration of a novel object and consumption of novel food (Liebl and Martin 2014).

Reactions of Finnish and Bohemian birds toward firebugs

The behavior of birds toward firebugs was affected by the firebug coloration. In contrast to behavior toward novel palatable prey, the Finnish birds partly differed from the Bohemian birds in their reactions. Nonaposematic firebugs were attacked in similar proportions by Finnish and Bohemian birds. The birds from both populations also hesitated about the same time before attacking nonaposematic firebugs, and learned to avoid them at a similar rate. In Finnish birds, the attack latencies for nonaposematic firebugs correlated with those for novel palatable prey, whereas in Bohemian birds the latencies were not correlated. These results indicate that nonaposematic, brown-painted firebugs were novel for Finnish birds, and that the reaction to them followed the general behavior of birds toward a novel prey. Noncorrelated latencies of Bohemian birds suggest that these birds may be experienced with some similar, nonaposematic but unpalatable true bugs from the wild (e.g., some species of Rhywarochromidae), and that they partly generalized their experience. On the other hand, the Bohemian birds attacked nonaposematic firebugs more frequently than the aposematic ones, which confirms the results of previous studies (Exnerová et al. 2003, 2006) suggesting that the characteristic red-and-black color pattern of firebugs facilitates their recognition by avian predators.

The Finnish and Bohemian great tits significantly differed in their behavior toward aposematic firebugs. Most Bohemian birds avoided them on sight, whereas most Finnish birds attacked at least 1 individual. Finnish birds generally behaved toward aposematic firebugs in a similar way as to the nonaposematic ones, and their initial reactions (before the first contact with firebug defense chemicals) were correlated with their reactions to palatable prey of novel appearance (blue crickets). The differences in behavior toward the aposematic firebugs did not follow the differences between the 2 populations in exploration and neophobia. Birds from both populations behaved similarly in response to a novel object and to a novel palatable prey, and the only difference—hesitation to feed in presence of a novel object—does not correspond with behavior toward the aposematic prey, because the Finnish birds were more neophobic. Therefore, the difference in avoidance between the wild-caught Finnish and Bohemian great tits is likely to be the result of individual learning and their different experiences in the wild.

Although the Finnish birds attacked aposematic firebugs more frequently than the Bohemian birds, it is interesting that the
Finnish birds stopped attacking them on average after only 1 trial. This means that the Finnish wild-caught birds learned to avoid the novel aposematic firebugs considerably faster than the conspecific naive hand-reared birds from Bohemia, which attacked on average 5 firebugs (Svádová et al. 2009) before learning to avoid them. This difference suggests that the Finnish wild-caught birds may have generalized their previous experience with some unpalatable defended prey, even of a different appearance than firebugs, and this may have increased their avoidance learning rate.

Alternatively, the difference may suggest an innate bias against conspicuous aposematic prey (Lindström et al. 1999a), which can speed up avoidance learning. Avoidance learning against an aposematic prey that is evolutionarily novel needs typically several unpleasant experiences before the novel prey is learned to be avoided (e.g., Mappes and Alatalo 1997; Lindström et al. 1999b). In previous studies, naive juvenile great tits did not hesitate longer before attacking aposematic than nonaposematic prey (Svádová et al. 2009), but they showed an innate bias against aposematic prey, when the nonaposematic (Lindström et al. 1999a) or less conspicuous (Fabricant et al. 2014) alternative prey was present. These 2 alternatives are not mutually exclusive, and the reactions observed in wild-caught birds are likely the result of an interaction between an innate bias and individual experience. Assessing the effect of both processes would, however, require further experiments with naive predators and the use of the prey novel for both populations.

For the nonaposematic firebugs, the learning rates were similar in Bohemian and Finnish birds, indicating that the populations do not differ in their ability to learn avoiding unpalatable prey. The Finnish birds, for which both firebug color forms were novel, learned to avoid red-and-black and brown-painted firebugs at a similar rate. This agrees with previous studies with great tits and defended conspicuous prey where learning rate between “typical” warning colors versus gray or brown did not differ (Ham et al. 2006; Svádová et al. 2009). Because our experiment was designed mainly to test the attack willingness toward the aposematic prey, rather than learning abilities, we are unable to make any strong conclusions about the general differences in learning abilities between populations. Furthermore, we compared an experienced population to a naive one. In the future, it would be interesting to compare learning abilities between populations by using a completely novel aposematic prey and/or naive birds.

Our results indicate a stronger avoidance of aposematic bugs by female great tits than by males. Similar results were obtained in a study where female bobwhites (Colinus virginianus) showed more aversion than males toward red- and orange-dyed food (Mastrotata and Mench 1994). A different study with the same species however, found no differences between the sexes in color aversion, presumably because the individuals used in both experiments differed in age (Mastrotata and Mench 1995). Color aversion in females, but not in males, seems to increase with age (Mastrotata and Mench 1994). A possible explanation is that females consume more insects than males, and they teach chicks to avoid toxic prey. Whether this is the case for great tits is a matter of future research.

Aposematic Heteroptera and other insects of Central Finland as potential models

Wild-caught great tits are potentially experienced with an unknown number of both palatable and unpalatable species of insects, both in Central Finland (around the 62°N latitude) and Bohemia. Unfortunately, there are no data available to compare the abundance and diversity of potential insect prey between the 2 areas. However, because the reactions of the birds toward the firebug were specific, we consider the evaluation of heteropteran faunas informative, as it compares the numbers of potential models the birds may be experienced with from the wild.

We have documented that the fauna of aposematic terrestrial Heteroptera of Central Finland is much less diverse than that of Central Europe both in absolute and relative number of species (30 vs. 156 species, 10% vs. 17% of total faunas, respectively). The Bohemian great tits are certainly experienced with Pyrrhocoris apterus due to the frequent occurrence of the bug, its aggregations and its ubiquitous host plants (Exnerová et al. 2006); these birds can be potentially experienced with other chemically protected red-and-black true bug species as well (Hotová Svádová et al. 2010). Although P. apterus does not occur in Central Finland (Rintala and Rinne 2010), we cannot exclude a priori the local occurrence of insect species that the birds would generalize with. However, the other Finnish similarly colored aposematic insects are either too rare (Corizus hyoscyami, Rhopalidae), or look too different (plant bugs, Miridae; burnet moths, Zygaenidae; ladybird beetles, Coccinellidae) to function as models for generalization with red-and-black firebugs. Moreover, recent analysis of Finnish Lepidoptera showed that only less than 5% of caterpillars are aposematic (Mappes et al. 2014). It is still possible that the negative experience with some of the above taxa may have played a role in the generalization of the firebug color patterns as great tits have been shown to generalize their learned avoidance among colors (red, yellow, and orange; Ham et al. 2006), among aposematic species (Hotová Svádová et al. 2013) and between bi-chromatic symbols (Ihalainen et al. 2008).

CONCLUSIONS

Even though a broader generalization of our results is limited by the study of only 2 populations, we have shown that conspecific birds from 2 geographically distant populations may express similar reactions to a prey which is novel for both of them. In our case, food neophobia was low in both populations, whereas the avoidance of aposematic prey was different between the populations, and could be explained by differences in experience. Bohemian birds have plenty of opportunities to meet firebugs in their natural environment and learn about their unpalatability; Finnish birds do not have such opportunity. Our results emphasize that naive birds’ tendency to attack novel aposematic prey may have important implications for range extensions of prey species. We can not tell whether the difference in avoidance was a result of Finnish birds’ lower experience with any aposematic prey, or whether the avoidance learning is prey specific. Our results indicate, however, that studies on aposematism and mimicry based on geographically distant conspecific populations can be compared and generalized.

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REFERENCES


Speed MP. 2000. Warning signals, receiver psychology and predator memory. Anim Behav. 60:269–278.


