

Original Article

Direction and strength of selection by predators for the color of the aposematic wood tiger moth

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Conventionally, predation is assumed to select for conspicuousness and uniformity of warning signals in aposematic (i.e., chemically defended and warning signaling) prey because this enhances predators' initial and learned avoidance. On the other hand, it has been suggested that both variation in the background where the signal is displayed as well as variation in predators' probability to attack defended prey may favor intermediate signals or relax selection for signal monomorphism. We studied the direction and strength of selection for the hind wing color (orange vs. red) of female *Parasemia plantaginis* moths. Birds found the moths aversive and avoided them by sight both in laboratory and field experiments. A laboratory experiment with great tits showed that birds can discriminate between orange and red wings and attack red females less than orange females. This directional selection should decrease variation in hind wing color. However, the hind wing color did not significantly affect the "survival" of dead specimens under natural field conditions in a multipredator community. In addition, even though both orange and red were highly conspicuous against green leaves and silver birch trunks (backgrounds used in the studies), the magnitude of avian-perceived chromatic contrast (conspicuousness) differed against these backgrounds. Our results suggest that depending on the signal environment (background, predator community) directional selection for the warning signal monomorphism can be relaxed. *Key words:* Arctiidae, conspicuousness, predator community, predator perception, warning signals. [*Behav Ecol* 22:580–587 (2011)]

INTRODUCTION

Aposematic animals have conspicuous warning color patterns informing predators that the individual is toxic, unpalatable, or otherwise unprofitable (Poulton 1890; Ruxton et al. 2004). Predators learn to avoid unpalatable prey faster if they are conspicuous rather than cryptic (Gittleman and Harvey 1980; Roper and Redston 1987; Alatalo and Mappes 1996; Gamberale-Stille and Tullberg 1999; Lindström, Alatalo, Mappes, et al. 1999; Lindstedt et al. 2008) and if they are clearly visible (high contrast) against their background (Roper and Redston 1987; Lindström, Alatalo, Mappes, Riipi, et al. 1999; Gamberale-Stille 2001; Prudic et al. 2007). In addition, conspicuousness may permit fewer recognition errors (Guilford 1986; Gamberale-Stille 2000). Many typical warning colors (orange, yellow, and red combined with black) are also innately aversive in comparison with common cryptic colors, such as green and brown, which can increase the benefits of being warning colored (Smith 1975; Schuler and Hesse 1985). Predators also learn 1 signal more easily than several signals (see e.g., Mallet and Barton 1989; Joron and Mallet 1998; Kapan 2001; Beatty et al. 2004; Rowland et al. 2007; but see Rowe et al. 2004; Ihalainen et al. 2007). Therefore, selection is expected to favor the most conspicuous and most common color morph and decrease variation in warning coloration.

Despite the advantages of clearly visible warning colors (see also Guilford 1986; Gamberale-Stille 2000 [recognition errors]; Schlenoff 1984; Marples et al. 1998; Thomas et al. 2003 [novelty effects]; Roper and Redston 1987; Roper 1990

[memorability]; and Sherratt and Beatty 2003; Ham et al. 2006 [distinguishable from edible prey] for other benefits of conspicuousness), intermediate or weak warning signals may be selected for instead of overtly conspicuous ones if the probability of predators attacking defended prey varies (Endler and Mappes 2004). For example, conspicuousness can make prey individuals more vulnerable to attacks by naïve (e.g., Lindström et al. 2001; Riipi et al. 2001; Lindstedt et al. 2008) and specialized (Yosef and Whitman 1992) predators. In addition, the predators visual cognitive and learning abilities as well as searching behaviors and resistance to prey defenses, may vary both within and among species, exposing aposematic prey to variable predation risk (Calvert 1979; Fink and Brower 1981; Yosef and Whitman 1992; Pinheiro 1996; Mappes and Alatalo 1997; Marples et al. 1998; Exnerová et al. 2003, 2007). Their vulnerability to predation may also depend on the amount of alternative prey available (Merilaita and Kaitala 2002; Lindström et al. 2004) and the hunger level of predators (Barnett et al. 2007; Sandre et al. 2010). Predation could therefore select for intermediate signals in some species (Endler and Mappes 2004; Tullberg et al. 2005; Ruxton et al. 2009) or allow for signal variation in others (Rowe et al. 2004; Darst et al. 2006; Ham et al. 2006; Ihalainen et al. 2007).

To evaluate the strength and direction of selection by predators on warning color patterns, it is important to (a) understand how predators see different pattern elements of prey coloration (Stevens 2007), (b) how they respond to the signals per se (variation in avoidance learning rate), and (c) what the effect of the signal is on the overall survival of prey in field conditions where the predator community is diverse with several predator species varying in experience, perception, hunger level, and learning ability (Endler and Mappes 2004). Here, we used these approaches to evaluate selection on variable hind wing coloration (continuous variation from

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Received 10 September 2010; revised 21 January 2010; accepted 7 February 2011.



Figure 1
Continuous variation in *Parasemia plantaginis* female color.

orange to red with black patterns) of wood tiger moth (*Parasemia plantaginis*, Arctiidae) females (Figure 1).

We first tested whether *Para. plantaginis* females are unprofitable as prey and whether bird predators could associate their conspicuous color with unprofitability. We offered *Para. plantaginis* females and cryptic edible wax moths (*Galleria* sp.) to pied flycatchers (*Ficedula hypoleuca*) in consecutive trials in the field in the vicinity of their nest boxes. Subsequently, we also compared the survival of dead *Para. plantaginis* females that varied in coloration with cryptic and palatable wax moths pinned to tree trunks in the field. During the field experiment, we also monitored the presence and behavior of avian predators in the study sites and appraise which species are likely to exert the most predation pressure on moths. *Parasemia plantaginis* larvae are known to be unpalatable to predators (Lindstedt et al. 2008) and the defense chemicals they sequester are transferred to the adult stages (Lindstedt et al. 2010). Therefore, we also expect *Para. plantaginis* females to be unprofitable as prey. Furthermore, yellow, orange, and red color patterns are shown to accelerate predator learning (Sillén-Tullberg 1985; Mappes and Alatalo 1997; Gamberale-Stille and Tullberg 1999; Exnerová et al. 2006), and we therefore expected *Para. plantaginis* females to be aposematic. If *Para. plantaginis* females are aposematic, predators should find them aversive and avoid them when repeatedly encountered (e.g., Ruxton et al. 2004).

In the second part of the study, we tested the possible fitness differences between orange and red females. Because the appearance of defensive coloration (conspicuousness) will depend on the predator's perception (e.g., Stevens 2007), we analyzed by using an avian vision model whether or not birds (blue tits, *Parus caeruleus*) can potentially discriminate between orange and red females, and how much the female morphs differ in conspicuousness against different natural backgrounds used in the predation experiments (Vorobyev and Osorio 1998). We also tested the signal function of the orange versus red hind wings in a learning experiment under laboratory conditions by using wild-caught great tits (*Parus major*) as predators. If the signal efficacy of the red and orange hind wings differs, we predicted that avoidance should be stronger for the more conspicuous color pattern against the test background. However, if avian predators are not able to discriminate between the different color forms or if the difference does not affect foraging behavior, it could relax selection toward color pattern monomorphism and conspicuousness and explain why the variation in female coloration is maintained.

Finally, we reanalyzed the survival data from the field predation experiment to evaluate possible survival differences of orange and red females when the predator community is more complex. Even if some predator species would cause directional selection toward one color, variation among predators in the field (in their experience, perception, cognitive skills, and hunger) could induce variation in predation pressure and relax directional selection (Endler and Mappes 2004).

METHODS

Prey species

All *Para. plantaginis* females were reared in a laboratory (e.g., Lindstedt et al. 2009) on a similar diet (lettuce for the first 10

days after hatching and, after that, only *Taraxacum* sp.) to ensure similar levels of diet-derived chemicals in the body. Adult individuals do not feed, thus, the larval diet determines the unprofitability of the individual. Based on a previous experiment, there is no indication that the defense chemical content of adult females would vary according to their coloration when their diet includes plant defense chemicals (Lindstedt et al. 2010). After the females hatched, they were killed by freezing and carefully dried or preserved in the freezer. Dried specimens were used in all experiments except the avoidance learning experiment with great tits, where frozen specimens were used (see below).

Hind wing color was categorized by human eye on a scale from 1 to 6, 1 being the yellowest, 2–3 orange, and 5–6 the reddest. Grade 1 females are rare and were not used in any of the experiments. When defense and signal conspicuousness of orange and red individuals were compared, we used individuals from grades 2–3 to represent orange signals and individuals from grades 5–6 to represent individuals with red signals. Hind wings that appear orange and red to humans differ both in brightness (orange is “lighter” than red) and hue when measured as reflectance (see Lindstedt et al. 2010). We also evaluated the color and luminance discriminability of orange and red females, and their contrasts against different backgrounds using a discrimination threshold model (Vorobyev and Osorio 1998) (see below).

To rehydrate the dried study specimens, they were pinned through the thorax to polystyrene and placed over a small amount of lukewarm water in an airtight container and left overnight. To expose the hind wings, moths were subsequently pinned through the thorax to polystyrene using 1.5 cm long haberdashery pins. A specimen pin was used at each wing joint to spread the forewing to a 90° angle, and again for the hind wing. Another specimen pin was then placed flat across the ventral surface of the wing and inserted into the polystyrene to hold the wing gently in place. Specimens were placed in an oven for 12 h on the lowest heat setting (50 °C). Finally, pins covering the wings were removed. Wings of the frozen females used in the avoidance learning experiment with great tits were carefully spread just before the experiment in the same position as the dried specimens in other experiments. It is possible that freezing and drying have decreased the defense chemical content (i.e., unpalatability) of the females. Thus, our measurements of unprofitability may be rather conservative.

As a cryptic control species, we used the wax moth *Galleria* (sp.) (Lepidoptera). Wax moths were fed on a diet containing honey, yeast, glycerin, and flour. They were slightly smaller than *Para. plantaginis*, and their coloration did not differ between sexes. Dried wax moths' wings were spread before the experiments in the same manner as *Para. plantaginis* females.

Predator species

The experiment with pied flycatchers was carried out at Konnevesi Research Station (lat 62°N, long 26°E) in Central Finland during 17–29 June 2006 in the vicinity of the nest-boxes at the beginning of the breeding season. Because female and male coloration of pied flycatchers differs, we were able to recognize birds individually from each nest-box. A feeding tray was placed approximately 2 m from the nest-box. Before the experiment, birds were familiarized with the feeding tray (20 × 30 cm) by offering them dead mealworms (Coleoptera: *Tenebrio molitor*, killed by freezing) ad libitum. The experiment was started after the nestlings were 2 days old, and at least one of the parental birds (female and/or male) had learned to feed from the feeding tray. We spent as little time as possible in the vicinity of the nest-boxes so as not to disturb the birds breeding. We tested a total of 15 birds.

Great tits ($N = 22$) used in the learning experiment were trapped from feeding sites around Konnevesi Research Station and were subsequently ringed for identification. Each bird was kept individually in an illuminated and air-conditioned plywood cage ($65 \times 65 \times 80$ cm, $w \times d \times h$) at approximately 15°C with a daily light period of 11.5 h. Each cage contained 3 perches for roosting and prey handling. Sunflower seeds, tallow, and fresh water were available ad libitum except prior to the experimental trials when the birds were food deprived for approximately 2 h. The experiment with great tits was performed outside breeding season between October and November 2007. After the experiments, the birds were released at their capture sites. All the birds remained in good health throughout their captivity. Experimenting with pied flycatchers and great tits was licensed by the Ethical Committee of the University of Jyväskylä (license numbers 19/22 May 2006 and 36/22 May 2006) and keeping great tits in captivity also by the Central Finland Environment Centre (number KSU-2007-L-425/254). All animal experimentation met the Association for the Study of Animal Behaviour guidelines for ethical treatment of animals.

Aposematic function of *Para. plantaginis* female color pattern

We tested whether *Para. plantaginis* females are unprofitable prey and whether their conspicuous coloration functions as a warning signal for pied flycatchers. During the experiment, we offered a total of 6 dead moths (3 *Para. plantaginis* females and 3 wax moths) per bird in a random order on a feeding tray in front of their nest-boxes. Birds were observed at a distance of 10–15 m with a telescope (Zeiss Diascope 85 T* FL, Germany). Attack risk (attacked or not) and attack latency (time between bird clearly observing the prey and attack) were recorded. Attack risk was scored “attacked” when the bird touched, ate, or removed the specimen and “no attack” when the bird clearly observed but did not touch the specimen. For the analyses, attack latency in no attack category was limited to a maximum of 80 s, which was the longest recorded time. Foraging motivation was tested after the moth presentations by offering the birds mealworms from the tray to ensure that no attack of the moths was not due to satiation.

Variation in conspicuousness

We used discrimination threshold modeling to compare the hue and luminance of the different hind wing colors. The discrimination threshold model used assumes that noise in the receptors limits discrimination ability (Vorobyev and Osorio 1998; Vorobyev et al. 1998). Thus, the model predicts when an animal can discriminate between 2 objects, and if so, how different the objects appear (Vorobyev et al. 1998). Discrimination threshold models utilize information about the visual system, such as the sensitivity and relative abundance of different receptor types, and estimates of noise that arise in the photoreceptors. The model can also provide discrimination thresholds for luminance.

First, the different hind wings and a sample of green leaves were measured with an Ocean Optics (Dunedin, FL) USB4000 spectrometer held at 45° to normal, with illumination by a PX-2 Pulsed Xenon Lamp, recorded in 1 nm intervals from 300 to 750 nm, expressed relative to a Spectralon 99% white reflectance standard (Labsphere, Congleton, United Kingdom). Color of the birch trunk (*Betula* spp.) was measured similarly except with AvaSpec-2048-SPU (Avantes, Broomfield, CO) spectrometer with illumination by an AvaLight DHS Deuterium-Halogen light source. Average spectra were taken for each stimulus type, followed by modeling a blue tit's photon catch

values for the single and double cones (Hart et al. 2000) with a standard D65 irradiance spectrum. Color vision in birds stems from the 4 single cone types (Cuthill 2006), whereas luminance-based tasks apparently stem from the double cones (Osorio and Vorobyev 2005). For the color model, we therefore used the 4 single cones, whereas the luminance model was based on the double cones (as Siddiqi et al. 2004). For the discrimination model, we used a Weber fraction of 0.05 for the most abundant cone type and the relative proportions of cone types in the blue tit retina (long wave = 1.00, medium wave = 0.99, shortwave = 0.71, and ultraviolet [UV] sensitive = 0.37). We took 5 measures of the same color per individual from 10 red females (belonging to grades 5–6) and 10 orange females (grades 2–3). For the results of the discrimination model, values (“just noticeable differences” or “JNDs”) of <1 are indistinguishable, values between 1 and 3 are hard to distinguish unless under optimal conditions, and values >5 are easy to tell apart under most conditions.

Hind wing color and predators' avoidance learning rate

We tested whether predators learn to avoid orange and red *Para. plantaginis* females at different rates: 10 great tits were tested with orange females and 12 great tits with red females. The avoidance learning experiment was conducted in cages (plywood, $50 \times 50 \times 70$ cm, $w \times d \times h$) at Konnevesi Research Station (see e.g., Ham et al. 2006). The cages contained a perch and a water bowl, and were lit with bird lamps (Arcadia Products plc, Redhill, United Kingdom) that also emitted UV light. Thus, the possible differences in the UV reflectance of the moth color patterns were also visible for the birds. Birds were observed through a small mesh-covered window on one side of the cage, and the cages were placed in a dark room so that the birds were less aware of the observer. Prey were offered through a hatch behind a visual barrier which enabled us to record the exact time of prey detection as the bird had to go round the barrier or fly on top of it to see the prey. The birds were first familiarized with the cage for 2 h by letting them forage on sunflower seeds from a white dish.

Parasemia plantaginis females were offered to the birds one at a time on a white dish on top of a fresh green leaf (*Alnus incana*) in 5 consecutive trials. Before the trials, the birds' motivation to feed was tested by offering them a mealworm. For all birds, we measured attack latency as the time from when the bird noticed the prey to when it touched the prey with its beak (attack). Prey were considered rejected if the bird did not attack it within 2 min from noticing it. If the bird dropped the moth after attacking or handling it, we waited for 2 min to allow the moth be eaten. Attack latency has been previously used to measure avoidance learning rate (Shettleworth 1972; Terrick et al. 1995; Marples and Roper 1997; Roper and Marples 1997; Gamberale-Stille 2000, 2001). We also recorded total number of moths attacked over the 5 trials. Because hunger can affect a predator's readiness to attack defended prey (Sherratt et al. 2004; Barnett et al. 2007; Sandre et al. 2010), we also measured the hunger level of the birds quantitatively by letting them eat mealworms from a petri dish freely for 5 min after the trials. We weighed the mealworms before and after and used the difference (mass of eaten mealworms) as a covariate when testing avoidance learning rates (Sandre et al. 2010).

Survival of *Para. plantaginis* females in the field

We studied the net benefits of *Para. plantaginis* female coloration (grades 2–6) compared with the cryptic and palatable wax moth by pinning dead specimens to birch tree trunks in the field. As we knew the coloration of each female, we also

made post hoc comparisons where we reanalyzed the difference in survival of orange (grades 2–3) and red females (grades 5–6). The experiment was conducted in Ruovesi, Finland between 25 August and 24 September 2006. Eleven different sites were used sequentially. Alternate *Para. plantagin*is females and wax moths ($N = 175$ for each species) were pinned at eye-level to silver birch trees (*B. pendula*, white parts of the trunk). We chose birch trees to minimize variation in the background color among the treatments and study sites. Birches were the most abundant trees providing relatively uniform background in all study areas. Birch trees are also common in the natural habitats of *Para. plantagin*is; the moths can be found resting on tree trunks in addition to grasses and rocks. Chosen trees had a diameter of 15–25 cm; they were of a generally uniform bark appearance and were a minimum of 30 m apart along a track. This was to keep the moths sufficiently far from each other to minimize predator learning or interaction; that is, birds could not be attracted to a cryptic moth by proximity to a warning colored moth. To minimize arthropod predation, we excluded trees with signs of ant and spider presence, and a 1 m circumference on the ground around the trunk was searched for patrolling ants. We followed predation of the moths for 5 days (recording them as “alive” or “attacked”) checking specimens at approximately the same time each day. In addition, bird species and their numbers were observed at the sites and observations of bird avoidance or attack were also made. *Parasemia plantagin*is is not common in the area and was likely to be novel for most local birds. However, birds, including juveniles, were likely to have had some experience of aposematic prey through hunting and foraging as it was early autumn.

Statistical analyses

To test the unprofitability of *Para. plantagin*is females and the warning function of their coloration, we analyzed the change in attack latencies of pied flycatchers between first, second, and third *Para. plantagin*is female and wax moth presentations, respectively. We used a nonparametric Wilcoxon test for pairwise comparisons between different encounters because it takes into account the dependent structure of the data. As a dependent variable between the trials, we used “change in attack latency” (i.e., avoidance learning rate) and prey type (*Para. plantagin*is female vs. wax moth) as a factor. We also analyzed the differences in the total number of attacked *Para. plantagin*is females and wax moths with a Wilcoxon test.

Differences in avoidance learning rates of great tits between orange and red females were tested with repeated measures analysis of variance. We analyzed the change in attack latencies (seconds) between the 5 consecutive trials of each bird (dependent variable); the color of the female (orange or red) was the between-subject factor. Hunger level (mass of eaten mealworms) was used as a covariate in the initial analysis, but it did not affect the avoidance learning rate ($F_{4,13} = 0.189$, $P = 0.940$) and was omitted from the final model. Similarly, hunger level as a covariate did not affect the total number of attacked prey which was analyzed using a generalized linear model ($F_{4,13} = 1.111$, $P = 0.393$).

For the field study, survival was analyzed with Cox proportional hazards regression (Cuthill et al. 2005) to identify significant predictors of bird attack using the variable prey type (*Para. Plantagin*is vs. wax moth; orange hind wing vs. red hind wing) and study site. Significance was tested with Wald statistics (W). We included only color classes 2–3 and 5–6 as these were also used in the learning experiment with great tits and the discrimination threshold model. Moths that survived until the fifth day were considered censored. Effect

sizes are odds ratios (OR), where a value of 1.00 indicates that 2 treatments have identical survival probabilities. All the analyses were run with SPSS 16 software.

RESULTS

Aposematic function of *Para. plantagin*is female color pattern

Pied flycatchers found *Para. plantagin*is females aversive in general (all presentations combined) as they hesitated longer (Wilcoxon signed-rank test: $Z = -2.574$, $P = 0.010$) and attacked fewer ($Z = -2.677$, $P = 0.007$) *Para. plantagin*is females than wax moths (Figure 2). For wax moths, attack latency did not change from first to second ($Z = 0.000$, $P = 1.000$), from second to third ($Z = -0.668$, $P = 0.504$), or from first to third encounter ($Z = -0.350$, $P = 0.726$). For *Para. plantagin*is females, attack latency did not differ between first and second encounter ($Z = -0.178$, $P = 0.859$), but it increased from second to third encounter ($Z = -2.207$, $P = 0.027$) and nearly significantly also from first to third encounter ($Z = -1.939$, $P = 0.052$). This suggests that pied flycatchers learned to avoid *Para. plantagin*is. However, variation among individual birds was high.

Variation in conspicuousness

The discrimination threshold model shows that avian predators should be able to discriminate between orange and red hind wing color (Table 1). The within pattern contrast (black vs. orange or red) is higher for red females compared with orange ones, even though patterns should be clearly visible in both color types (Table 1).

Contrast between the prey hue and background (green leaf) was higher for red individuals than for orange individuals (Table 1), although this difference was very small and both types are highly contrastive. Thus, in learning experiments, where moths were offered to birds against the green leaf, red females were more contrasting in terms of chromatic (hue) contrast. Both colors were also highly conspicuous against white birch trunks (Table 1), although orange individuals had slightly higher chromatic contrast. Similarly, both orange and red females are easy for birds to see in terms of luminance against both background types (green and white)

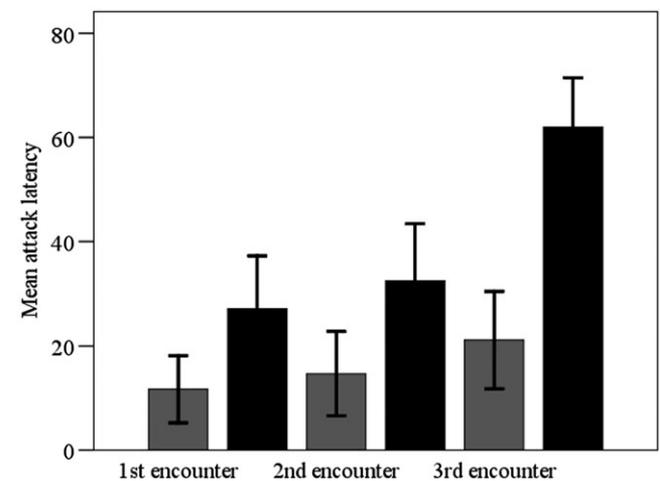


Figure 2

Mean attack latencies (seconds, ± 1 standard error [SE]) for 1st, 2nd, and 3rd encounters by pied flycatchers for wax moth controls (gray bars) and *Parasemia plantagin*is females (black bars).

Table 1
Discrimination values for color (hue) and luminance according to model by Vorobyev et al. 1998

Comparisons of signal and background	Discrimination value
Color	
Orange versus red	4.87
Orange versus black	13.81
Red versus black	17.58
Orange against green leaf (<i>Alnus incana</i>)	27.27
Orange against birch trunk (<i>Betula pendula</i>)	23.14
Red against green leaf (<i>Al. incana</i>)	29.55
Red against birch trunk (<i>B. pendula</i>)	21.07
Luminance	
Orange versus red	6.45
Orange versus black	12.65
Red versus black	8.61
Orange against green leaf (<i>Al. incana</i>)	33.28
Orange against birch trunk (<i>B. pendula</i>)	25.75
Red against green leaf (<i>Al. incana</i>)	22.78
Red against birch trunk (<i>B. pendula</i>)	15.25

Values >5 are easy to tell apart in most conditions.

(Table 1). Orange individuals may appear brighter (measured as luminance) against green leaves and white backgrounds possibly increasing their detectability from longer distances (Table 1).

Hind wing color and predators' avoidance learning rate

There was no avoidance learning of *Para. plantaginis* females by great tits for either orange or red signals as the mean attack latency did not change from trial to trial ($F_{4,15} = 0.437$, $P = 0.780$); there was also no learning rate by female color interaction ($F_{4,15} = 1.126$, $P = 0.345$) indicating that "no learning" trend was similar for both colors (Figure 3). However, red females were generally better protected than orange ones, as great tits attacked less red females overall ($Z = -2.584$, $N = 12$, $P = 0.010$) and constantly hesitated longer before attacking them ($F_{1,18} = 6.632$, $P = 0.019$) (Figure 3), suggesting great tits had innate or learned bias for red females. Great tits always attacked and ate mealworms offered in the begin-

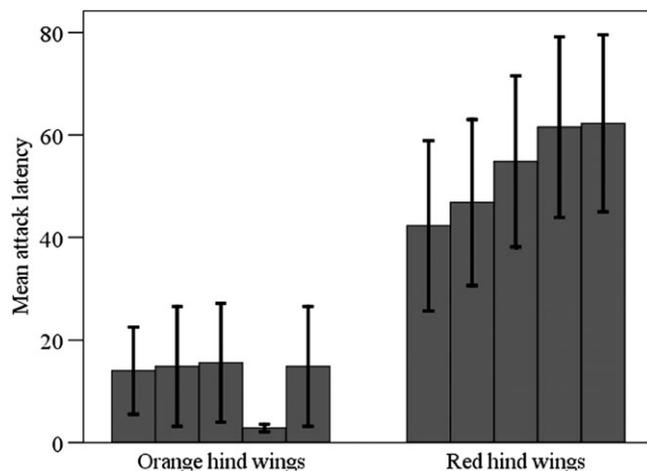


Figure 3
 Mean attack latencies (seconds) by great tits to orange and red *Parasemia plantaginis* females.

ning of the learning trial, and hunger level did not affect the behavior of the birds (see Statistical analyses).

Survival of *Para. plantaginis* females in the field

Parasemia plantaginis females survived better than the cryptic wax moths; 19% of *Para. Plantaginis* females and 38% of wax moths were attacked over the 5-day period (Figure 4). *Parasemia Plantaginis* also survived longer (mean number of days until attacked: *Para. Plantaginis* 1.941, standard deviation [SD] = 1.043; wax moth 1.561, SD = 0.787) ($W_1 = 7.843$, $P = 0.005$, OR = 3.457). The survival differed also among study sites ($W_1 = 6.067$, $P = 0.014$, OR = 0.865), but there was no significant interaction between the study site and survival of the prey type ($W_1 = 1.256$, $P = 0.262$, OR = 0.921).

Orange females were attacked 10% more than red females; 23% of the orange females and 13% of the red females were attacked over the 5-day period (Figure 5). Seventy-eight percentage of orange ($n = 62$, 95% confidence interval [CI] = 69–89%) and 88% of red females ($n = 113$, 95% CI = 81–96%) are estimated to survive until day 5. However, the Wald statistics show that this difference is not significant. Neither color ($W_1 = 0.609$, $P = 0.435$, OR = 1.783), the study site ($W_1 = 3.049$, $P = 0.089$, OR = 0.875), nor the interaction between study site and coloration ($W_1 = 0.138$, $P = 0.710$, OR = 0.956) have a significant effect on survival.

DISCUSSION

Predation by visually hunting predators is one of the most important selective pressures shaping the evolution of color patterns in prey (Fisher 1930; Cott 1940; Edmunds 1974; Ender 1991). Predators could be expected to select for conspicuous (Gittleman and Harvey 1980; Gamberale-Stille and Tullberg 1999; Lindström, Alatalo, Mappes, Riipi et al. 1999; Riipi et al. 2001; Lindstedt et al. 2008) and uniform (Müller 1879; Kapan 2001; Beatty et al. 2004; Rowland et al. 2007) warning signals in aposematic prey. However, some species can also benefit from moderate signals (see Darst et al. 2006 for poison arrow frogs) and in others, including, for example, the *Para. plantaginis* moth (Ojala et al. 2007; Lindstedt et al. 2008) or *Harmonia* lady beetles (Bezzerides et al. 2007), warning signal expression can vary.

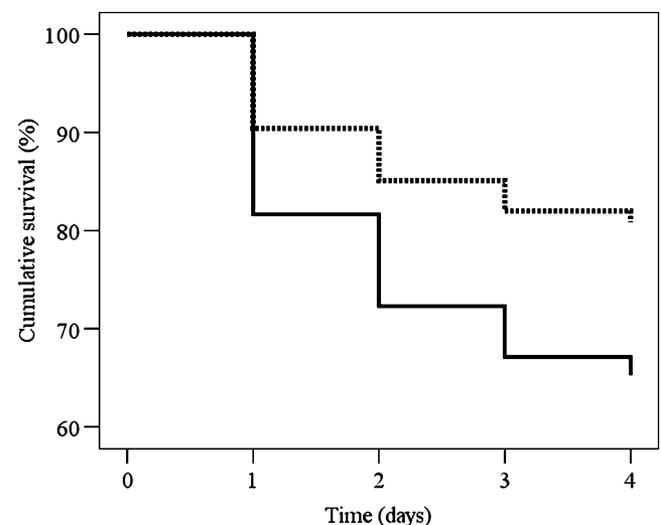


Figure 4
 Cumulative survival of conspicuously colored *Parasemia plantaginis* females (dashed line) and cryptic wax moths (solid line) in the field.

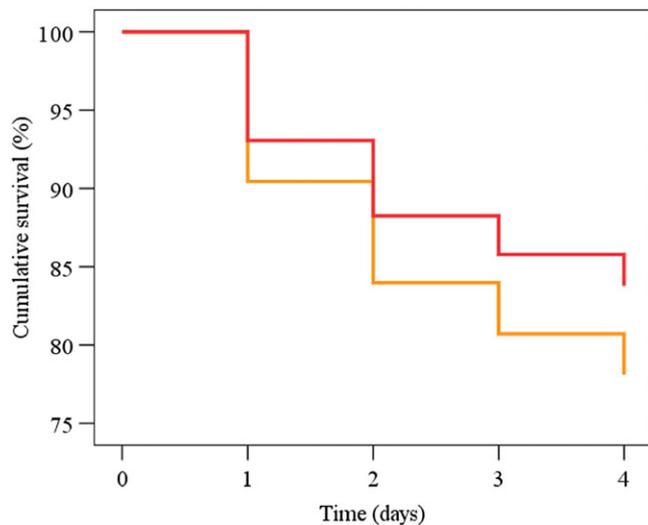


Figure 5
Cumulative survival of orange (orange line) and red (red line) *Parasemia plantaginis* females in the field.

In this study, we first confirmed that *Para. plantaginis* females are unprofitable prey to avian predators and that their appearance, with red/orange and black patterning (Figure 1), function as warning signals: despite individual variation in behavior, pied flycatchers learned to reject *Para. plantaginis* females by sight while favoring wax moths as prey (Figure 2). The control wax moths were also attacked more frequently than *Para. plantaginis* in the field. Great tits did not show avoidance learning as their latency to attack female moths did not increase from trial to trial. However, in the experiment birds were not presented with alternative edible prey, which may have increased their willingness to attack unprofitable prey. Taken together, the *Para. plantaginis* females seem to be moderately defended, and their unprofitability does not deter all predators, which is also the case with *Para. plantaginis* larvae (Lindstedt et al. 2008).

Our results also suggest that selection by predators should decrease the variation in signal expression in *Para. plantaginis* females. The discrimination threshold model predicted that birds are able to discriminate between the orange and red extremes of hind wing colors, at least in good lighting (Table 1). In concordance, great tits did attack orange and red females at different rates in the laboratory, which shows that birds do perceive the color difference (Figure 3). Because high achromatic (luminance) contrast makes objects more conspicuous from a distance (Osorio et al. 1999), orange females could be easier for predators to detect against the green backgrounds (Table 1) where the females most often occur in the wild (Ihalainen E, personal observation). On the other hand, the chromatic component of the signal (contrast in hue) is easier for predators to learn than the achromatic component of the signal (Osorio et al. 1999). Therefore, red, that has higher chromatic contrast against green (Table 1), should be an easier stimulus to learn. In the laboratory, the birds did indeed prefer orange females over red females, when presented on a green leaf (Figure 3). However, the benefit of darker red hind wings seems to be due to the predators' innate wariness or previous experience in the wild because the great tits did not show significant avoidance learning for either of the female colors but attacked less red individuals overall and hesitated longer before touching them (see also Gamberale-Stille and Tullberg 1999). Nonetheless, this difference in predator behavior should cause directional

selection toward darker red hind wings (see also Gamberale-Stille and Tullberg 1999).

In the field, the survival of orange and red females did not differ significantly, even though the overall survival of red females was 10% higher than orange females. Our results therefore propose that evaluating the fitness of red and orange females against one predator species (great tits) may overestimate the overall benefits of red signals in the wild where the predator community is more complex and viewing distance and lighting varies. For example, we note that according to the discrimination threshold model, red and orange wing colors may look more or less the same to predators under suboptimal conditions, for example, in poor lighting or against certain backgrounds where the contrasts are lower. Also, both orange and red were highly conspicuous against birch trunks (orange more than red) (Table 1), suggesting that in terms of conspicuousness, both signals can be effective warning signals against predators. This is also supported by similar work by Stevens et al. 2009 investigating bird avoidance of high contrast "eyespot" on artificial prey in the field, as they found no difference between treatments (red, yellow, or blue spots) of approximately equal contrast. Furthermore, efficient signal may provide little additional protection if the predator is immune to the prey's chemical defenses, if the predator has forgotten the warning signal, is naïve, or simply hungry (Mappes et al. 2005). However, conclusions from the reanalyzed field data should be drawn with caution because the experiment was not specifically designed to test the mortality risk of orange and red females in the field. Thus, further study is needed to perform controlled tests for the effect of background and color contrast variation for signal function.

Predator-prey experiments addressing the evolution of aposematism often focus on 1 or 2 predator species (but see Exnerová et al. 2003, 2007). In doing so, behavioral variation can be limited and complex interactions are simplified (which, of course, is the purpose of controlled laboratory experiments). There is also little available information on the frequency of attacks and diversity of predator species that attack aposematic prey. This kind of natural history data would help to evaluate the net benefits of aposematism in the wild. We recorded a total of 28 bird species across the 11 sites (see Supplementary Material) yet only a few species were well represented. The genera *Corvus* (crows) and *Parus* (tits) were widely distributed across all sites and frequently found in high numbers. *Pica pica* (magpie), *Motacilla alba* (pied wagtail), *Erithacus rubecula* (robin), and *Apus apus* (swift) were also widely distributed and numerous. According to our observations tits, robins and magpies were responsible for most of the predation of both cryptic and aposematic moths (Supplementary Material). For example, based on our observations robins exhibited a foraging strategy that consisted of inspection of the central part of the trunk for most birch trees in a small area, repeated daily, with multiple individuals inspecting each tree. When present, we expected robins to be voracious predators of both cryptic and aposematic moths (see Supplementary Material). We do not know the foraging history of the individual birds in the laboratory and field studies, but overall, it seems that also experienced predators can attack aposematic prey at least when they are easily available. Predation risk may also vary significantly locally and temporally possibly causing spatiotemporal variation in the benefits of expressing conspicuous signals (see also Endler and Mappes 2004; Mappes et al. 2005).

Variation in vulnerability to predators could make it beneficial for aposematic prey to have color pattern that is conspicuous for the closer distances but cryptic at a longer distances (Edmunds 1974; Marshall 2000; Tullberg et al. 2005). In *Para. plantaginis* females, only the hind wings are brightly colored,

whereas the color pattern of the forewings is less conspicuous and could have a disruptive function (Figure 1). Especially when resting, females keep their hind wings partly hidden and display them fully only when flying or disturbed (Lindstedt C, personal observation). Thus, it is possible that their color pattern has both a cryptic and warning signal function, and the relative importance of these functions may vary weakening the directional selection toward conspicuous red patterns in the hind wings of females.

Moreover, although predation and predator community structure alone can have complex effects on the fitness of aposematic prey (Mappes et al. 2005 and results from the present study), other selective forces, such as defense against pathogens (Friman et al. 2009), thermoregulation (Lindstedt et al. 2009), and diet quality (Grill and Moore 1998; Ojala et al. 2007; Lindstedt et al. 2010) can also affect the strength and direction of warning signal expression through trade-offs. *Parasemia plantaginis* females do not sequester diet-derived iridoid glycosides but excrete them keeping the iridoid glycosides levels in their body constant across diets (Lindstedt et al. 2010). Excretion is costly; it decreases fertility and constrains warning signal efficacy. Individuals on a diet high in iridoid glycoside content (greater need for excretion) have a light orange coloration as opposed to the dark red coloration of individuals on low iridoid glycoside diets (lower excretion costs; Lindstedt et al. 2010). Thus, even though red coloration would be more effective against predators, detoxification and/or excretion costs of their host plant's chemical defenses could induce variation in signal expression depending on, for example, the availability and abundance of host plants that contain iridoid glycosides. Together these multiple ecological interactions can shape the evolution of warning signals and maintain diversity in aposematic displays.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behaveco.oxfordjournals.org/>.

FUNDING

Academy of Finland (Finnish Centre of Excellence in Evolutionary Ecology and Evolutionary Research) and Ella and Georg Ehrnrooth foundation. Martin Stevens was supported by a Biotechnology and Biological Sciences Research Council David Phillips Research Fellowship (BB/G022887/1).

We thank Markus Rantala for providing the wax moths for our study, Tuire Pehkonen for assistance in the lab, Ossi Nokelainen for assistance with reflectance measurements, and Helinä Nisu for taking care of the birds. Jaakko Heikurinen kindly helped to identify birds in the field.

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