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Changes in predator community structure shifts the efficacy of two warning signals in Arctiid moths

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Summary

1. Polymorphism in warning coloration is puzzling because positive frequency-dependent selection by predators is expected to promote monomorphic warning signals in defended prey.

2. We studied predation on the warning-coloured wood tiger moth (*Parasemia plantaginis*) by using artificial prey resembling white and yellow male colour morphs in five separate populations with different naturally occurring morph frequencies.

3. We tested whether predation favours one of the colour morphs over the other and whether that is influenced either by local, natural colour morph frequencies or predator community composition.

4. We found that yellow specimens were attacked less than white ones regardless of the local frequency of the morphs indicating frequency-independent selection, but predation did depend on predator community composition: yellows suffered less attacks when Paridae were abundant, whereas whites suffered less attacks when Prunellidae were abundant.

5. Our results suggest that spatial heterogeneity in predator community composition can generate a geographical mosaic of selection facilitating the evolution of polymorphic warning signals. This is the first time this mechanism gains experimental support. Altogether, this study sheds light on the evolution of adaptive coloration in heterogeneous environments.

Key-words: aposematism, colour polymorphism, frequency-dependent selection, *Parasemia plantaginis*, predator-prey interactions

Introduction

Colour polymorphism (i.e. coexistence of individuals with sharply distinct colorations in a population) provides a great opportunity to study evolutionary processes maintaining genetic variation in the wild (Huxley 1955; Ford 1965), because morphs with lower fitness should go extinct (McKinnon & Pierotti 2010), unless there is conjunct selection maintaining equivalent net benefits among morphs (Gray & McKinnon 2007). Maintenance of colour polymorphism is often attributed to negative frequency-dependent selection (e.g. Sinervo & Lively 1996; Rueffler *et al.* 2006), local adaptations of genotypes (Mallet & Joron 1999; Calsbeek & Smith 2007) or deviating selective regimes due to environmental heterogeneity (Sandoval & Nosil 2005; Bell 2010). Despite the geographical mosaic of co-evolution theory (Thompson 2005) predicts that selection mosaic among populations can favour different evolutionary trajectories within a species (Thompson 1999), maintenance of colour polymorphism via local predator community structure has received surprisingly little experimental attention.

Colour polymorphism is particularly puzzling in aposematic species, which use coloration as warning signal to advertise unprofitability (e.g. aggressiveness, spines, toxins) to predators (Poulton 1890; Cott 1940; Ruxton, Sherratt & Speed 2004). This is because positive frequency-dependent selection should favour monomorphic warning coloration due to shared cost of predator education (Mallet & Barton 1989; Lindström *et al.* 2001; Beatty, Beirinckx & Sherratt 2004; Noonan & Comeault 2009; Chouteau & Angers 2012). For example, bird predators have learned to avoid the most common local colour morph (either green or blue) of a chemically defended leaf beetle *Oreina gloriosa*, depending on the respective subpopulation (Borer *et al.* 2010). The puzzle, however, is why populations still contain individuals of the less adaptive colour despite the strong selection against the rare

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morph (e.g. Mallet & Joron 1999; Mochida 2011). One possibility is that migration (i.e. gene flow) between subpopulations obstructs local adaptation, allowing less adaptive phenotypes to persist in low frequencies (Slatkin 1987; O'Hara 2005; Harley *et al.* 2006).

Importantly, warning signal uniformity between conspecifics is not the only factor affecting protection from predation (reviewed by Mallet & Joron 1999). Environmental heterogeneity can influence how predators treat warning signals in different contexts (Bond & Kamil 2006; Mallet 2010). For example, heterogeneity in the visual environment due to varying light conditions can alter how predators perceive prey appearance (Endler 1991) and thus how they associate appearance with prey quality. Nutritional condition of both the prey and the predator can further affect how profitable predators regard the prey (Barnett, Bateson & Rowe 2007) and therefore how likely they are to attack. Moreover, the efficacy of a warning signal likely depends on what kinds of predators are frequently present (Endler & Mappes 2004; Endler & Rojas 2009) and how they generalize different warning signals (Guilford & Dawkins 1991; Maynard-Smith & Harper 2003). Therefore, local predator communities could select for different warning colour patterns facilitating colour polymorphism.

The aposematic and polymorphic wood tiger moth (*Parasemia plantaginis*) males exhibit heritable white or yellow hind wing colour pigmentation with a varying degree of melanization (Nokelainen *et al.* 2012; Hegna *et al.* 2013; Nokelainen, Lindstedt & Mappes 2013), which makes them ideal to study maintenance of colour polymorphism in aposematic organisms. By using artificial prey resembling the white and yellow colour morphs (Fig. 1), we conducted a set of field experiments in five geographical locations where *P. plantaginis* occurs naturally with different frequencies of the two male morphs.

First, we study whether attack risk is frequency-dependent. We hypothesize that attack risk of the artificial prey types depends on the natural frequencies of *P. plantaginis* colour morphs: If selection for warning signals is positively frequency-dependent, as predicted according to the signal theory (e.g. Guilford & Dawkins 1991; Maynard-Smith & Harper 2003; Beatty, Beirinckx & Sherratt 2004), we can expect the locally rare morphs to be at a disadvantage. This should result in increased attack rates towards the locally rare colour morph (i.e. white disadvantage in yellow-dominated populations and *vice versa*). Alternatively, if attack risk does not depend on the natural frequencies of colour morph, then it would indicate frequency independence.

Secondly, we study the effect of varying bird predator community on morph attacks. We hypothesize that spatially varying bird predator community and habitat type can affect attack risk of the colour morphs (*rationale* Endler & Mappes 2004): If some bird species are prone to be bolder than others in attacking chemically defended species (Exnerová *et al.* 2003), then we can expect more conspicuous warning signal (Nokelainen *et al.* 2012) being less protective when such species are common in the bird community and *vice versa*. If in some habitats moths are more prone to attacks due to visibility costs, then it is possible that some habitats are more vulnerable to colour morphs than others. Alternatively, predator community or habitat does not affect survival of colour morphs differentially, which would indicate locally similar selection pressure to morphs by predators. In sum, this study sheds light on how local predator–prey interactions can facilitate colour polymorphism in aposematic species and how variation in adaptive coloration overall is maintained in heterogeneous environments.

Materials and methods

CRAFTING ARTIFICIAL PREY

In order to estimate the attack risk on wood tiger moth colour morphs in the wild, we crafted artificial moths with plasticine bodies and paper wings to resemble real *P. plantaginis* males (Fig. 1). The resemblance of artificial moths was ensured by using digital photographs of real wings, which we took of real dead specimens of *P. plantaginis* males with a Fujifilm Finepix S3 Pro UVIR digital camera (Fuji Photo Film USA Inc., Edison, NJ, USA). The wing photographs were adjusted to real *P. plantaginis* wing size (forewing length = 1·66 cm, body length = 1·30 cm)

Fig. 1. Wood tiger moths: First row (a) shows variation in both colour and melanisation among wood tiger moth male morphs. Notice that these specimens are pale museum samples, which wings have been spread out. Second row (b) shows artificial prey representing different male morphs used in predation experiment.

with GIMP 2 software (GNU Image manipulation program) and printed (HP Color LaserJet CP2025) on all-weather writing paper (Rite in Rain ©, JL Darling Corporation, Tacoma, WA, USA). The same wing image was printed on both sides to achieve uniform finishing of the artificial moths. Finally, the wings were cut off from paper and the artificial moth was completed with a black plasticine body (Caran D'Ache, Modela Noir, 0259·005), which was attached on the wings with two-sided tape (Scotch) and an insect pin (Sphinx). To represent the natural colour morphs occurring in the experimental areas, we photographed hind wings with either white or yellow colour pigmentation, and either smaller (*c.* 40%) or larger (*c.* 60%) melanized (black) pattern. The wing photographs used for the artificial moths were randomly selected from a group of representative samples of each colour morph, but only one wing of each morph was used to create the corresponding artificial prey type. Colour pigmentation of forewings was controlled, by distributing forewings with either yellowish or whitish colours equally across the treatments. Forewing melanization varies less than hind wing melanization, so we kept the black forewing pattern constant in all treatments resembling *P. plantaginis*.

We selected the artificial moths with the closest resemblance to the real moths. First, we printed several versions of artificial prey and then used the closest match to the real specimens. The match between the artificial prey and the real moth against green background was based on obtained contrast values in hue and luminosity (see further Hegna *et al.* 2013; Table S1, Supporting information). Because the colour morphs vary in conspicuousness (Nokelainen *et al.* 2012), we controlled for visibility of the artificial moth by presenting them on standard backgrounds, which were made of cardboard (i.e. coaster plate, diameter = 10 cm) and painted green (Helmi, M384, Tikkurila Oyj, Vantaa, Finland). The conspicuousness of the artificial moths against the green background was determined with an avian vision model (Vorobyev & Osorio 1998; Vorobyev *et al.* 2001). We took five spectral measurements (range 300–700 nm) per sample from the yellow and white areas of the hind wings from real specimens ($n = 5$), artificial moths ($n = 5$) and backgrounds ($n = 5$) with an AvaSpec-2048-SPU (Avantes, Inc., Broomfield, CO, USA) spectrometer with illumination by AvaLight DHS Deuterium–Halogen light source. UV range was included because, unlike humans, birds see UV wavelengths (Cuthill 2006). Average spectra were then used for the model, which estimated (based on known retinal sensitivity) a representative predator's (blue tit, *Cyanistes caeruleus*) perception of the colours as photon catch values for single and double cones (Hart, Partridge & Cuthill 2000), with a standard D65 irradiance spectrum. Four single cones were used for modelling the perception of hue, whereas the luminance model was based on double cones, because bird colour vision stems from four single-cone types (Cuthill 2006), and luminance discrimination is connected to double cones (Osorio & Vorobyev 2005). For the discrimination model estimating the perceived contrast between the moth and background colour (measured in units of just noticeable differences or 'JNDs'), 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, short wave = 0·71 and uv sensitive = 0·37). We also confirmed directly the similarity of the real moths to the artificial prey with spectrophotometer.

To account for the possibility that attack rates on the artificial *P. plantaginis* moths could be very low, we crafted a uniformly coloured control moth which would have allowed us to discern whether such low attack rates were due to general avoidance of all artificial moths irrespective of colour or whether attack rates were low on the warning-coloured prey only (possibly due to an unusually strong, artificial warning signal). Control moths were crafted under the same protocol as others, but their appearance was based on the wing colour of the wax moth (*Galleria mellonella*). Only a small sample from a real wax moth wing was copied and multiplied to cover the artificial wings, creating a uniform non-warning appearance. The control moths did not resemble any known species, and therefore, the predators could not possess learned avoidance towards them. However, all morphs were attacked, and hence, the artificial *P. plantaginis* moths were realistic enough to detect avian predation pressure. Artificial *P. plantaginis* were also attacked at the same rate as the controls ($Z = 0{\cdot}584$, $\mathrm{SE} = 0{\cdot}137$, $P = 0{\cdot}559$), which indicates that the printed warning signal was not overly 'frightening' or unnatural. Thus, we excluded the control moths from the further analysis.

PREDATION EXPERIMENTS

A set of field experiments was conducted in five geographical locations in 2010: Estonia (58°N, 25°E), Central Finland (62°N, 26°E), Southern Finland (60°N, 23°E), Åland (60°N, 20°E) and Scotland (57°N, 2°W). A total of 15 transects were set in each location (except in Åland there were 16 transects), so 76 transects in total 10 specimens of each morph (white less melanized, white more melanized, yellow less melanized, yellow more melanized and control; 50 artificial moths in total) were pinned in turns in 20 m intervals on 67 transects. However, in nine transects (all in Central Finland), controls were not used (resulting in 40 moths/transect). Artificial moths were presented on green backgrounds, which were mounted on 30 to 50-cm-tall stakes and placed on visible spots at the shrub level, enabling visually hunting predators (i.e. birds) to find them easily.

Artificial moths were determined 'attacked' if we observed clear damage to the plasticine body. Because we were interested in predation by visually hunting predators, only the attacks by avian predators were recorded. Avian attacks were identified by characteristically U- or V-shaped beak marks. We defined untouched individuals as 'survived'. Transects were in the field for 5 days. The artificial moths were checked every 24 h, and attacked or damaged specimens were replaced to keep the proportional hazards constant, but only the first-attacked specimens on each spot in the transect were used in the data. All experiments were conducted in the beginning of the natural flying period of *P. plantaginis* in early June.

THE EFFECT OF COLOUR MORPH FREQUENCIES ON PREDATION

Natural frequencies of male *P. plantaginis* colour morphs were monitored to obtain background data for our expectations of the direction of selection. Nets and 'pheromone traps' were used to capture males. Females from a laboratory stock were used as decoys producing the pheromone to attract the males. According to field surveys carried out in 2009–2011, the natural frequencies (%) of white and yellow colour morphs were as follows: Estonia 97 : 3 ($n = 123$), Central Finland 79 : 21 ($n = 177$), Southern Finland 44 : 56 ($n = 211$), Åland 35 : 65 ($n = 127$) and Scotland 0 : 100 ($n = 60$), respectively (Fig. 2). The frequencies remained fairly similar throughout the study years (Nokelainen, O., Hegna,

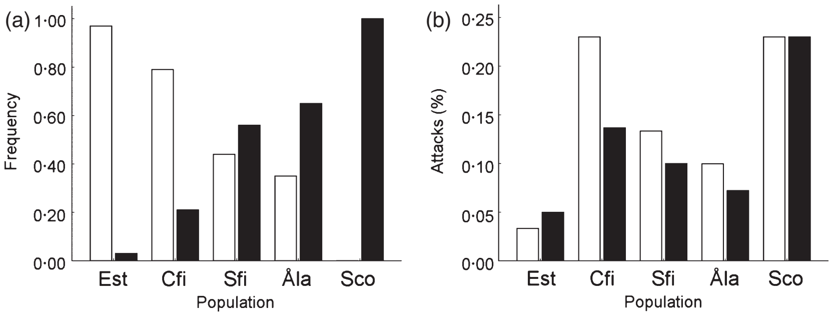

Fig. 2. Natural colour morph frequencies and attack risk of artificial moths in the experimental locations. For both figures: Est = Estonia, Cfi = Central Finland, Sfi = Southern Finland, Åla = Åland, and Sco = Scotland. White bars represent the white morph and black bars represent the yellow morph. (a) Natural frequencies of male wood tiger moth colour morphs in experimental locations according to field surveys carried out in 2009-2011. On *x*-axis the location, on *y*-axis frequencies of the colour morphs. (b) Observed attack risk of the artificial moths resembling the real wood tiger moth males in experimental locations after 5 days. On *x*-axis the location, on *y*-axis the attack proportions of colour morphs.

R.H., Valkonen, J., Lindstedt, C. & Mappes, J., unpublished). These locations represent both polymorphic and monomorphic populations, allowing us to study the role of frequency-dependent selection as a mechanism driving colour polymorphism.

THE EFFECT OF PREDATOR COMMUNITY ON MORPH SURVIVAL

To test whether environmental heterogeneity is causing divergence in selective regimes, we recorded the habitat type and predator community composition at the field sites. The biotope in which each artificial moth was presented was simply categorized as open area, bush or forest.

To estimate local predator community composition, we counted birds from the experimental transects using transect counting method (Sutherland 2006). Monitoring was done in good weather conditions from 04:00 to 12:00, when birds were active. All birds within a 50-m-wide 'research transect' (i.e. 25 m to the left and right from the observer) were counted and first identified to species level. Birds observed to be further than 25 m from the observer were excluded to make sure that birds represent the most potential predators in the vicinity of the transects.

As Passeriformes are logical predators for Lepidopterans, all other avian genera were excluded from analysis (e.g. hawks and waterfowl). Observed bird individuals were then grouped into family level (Svensson, Mullarney & Zetterström 2009), which conveniently represents functional groups of bird behaviour and foraging tactics (Table S2, Supporting information). We further excluded the least potential passerine predators, which are not adapted to prey on moths (e.g. crossbills) and concentrated only on relevant insectivores as the most potential predators (see the list of observations, Table S3 and Table S4, Supporting information). Finally, nine genera were used in the principal component analysis (see Statistical Methods section, Table S5, Supporting information).

STATISTICAL METHODS

For all analyses of attack risk, we used generalized linear mixed models (GLMMs) with a binomial response variable (survived or attacked) and a logit link function (equivalent to logistic regression). The models were fitted by Laplace approximation using the lmer function in R PACKAGE LME4 (Bates & Maechler 2009) with R version 2.12.2 (R Development Core Team 2009). Model selection was based on the smallest Akaike Information Criterion value. However, we seized all main effects in the final model to include all the key variables of interest. The results remained rather similar if a traditional backward stepwise protocol based on significant departure from chi-square distribution was applied. All fitted models incorporate location and transect as independent random factors to account for the sampling structure. From the best-fitting model to our data, we obtained predicted attack risk for both morphs.

Table 1. The effect of colour morph frequencies on predation: GLMM predicts the attack risk in relation to colour of the artificial moth (*morph*), the natural frequency of the yellow morph (in comparison with white) in the population obtained from the field surveys (*frequency*) and their interaction. The spatial effects of transects are nested within population and incorporated as random effects in the intercept

Source	Estimate	SE	*Z*	*P*
(Intercept)^a	−2·497	0·446	−5·618	**< 0·001**
Morph [yellow]	−0·481	0·220	−2·183	**0·029**
Frequency	1·079	0·735	1·467	0·142
Morph*frequency	0·384	0·326	1·177	0·239

Significant *P* values are denoted in bold. ^{a}intercept includes factor level: morph [white].

To analyse predation on different colour morphs, we performed separate analyses to specifically test two questions with biologically relevant interactions. We analysed whether (i) natural frequencies of colour morphs influenced on predation pressure and (ii) variation in predator community promotes diversity in warning signals.

To test for frequency-dependent selection on the colour morphs (white, yellow), we included as fixed factors the colour of the artificial moth, the natural proportion of the yellow morph in the *P. plantaginis* population obtained from the field surveys and their interaction (Table 1). There was no need to account for the frequency of the other morph, as it was inherently correlated with the proportion of the first morph. Melanization was not included in the first analysis to ensure direct comparison of colour morph with its respective natural frequency.

The bird community structures were comparable only among Northern European sites. The data from Scotland were not comparable, due to differences in species composition forming the family groups (e.g. Paridae, see Tables S3 and S4, Supporting information), and were thus excluded from the community models. Before analysing the effect of predator community composition, predator data were condensed with a principal component analysis (Table S5, Supporting information). This was necessary to generate variables to represent community composition rather than separate species or groups. Principal component analysis yielded three principal components (PCs) as a measure of predator community composition. First (describing the pipit–wagtail group, thrushes, warblers, flycatchers and crows), second (describing tits and shrikes) and third (describing accentors and starlings) principal components explained 52·9% of the variation in predator community (PC1 explained 24·6% of variation with eigenvalue 2·216, PC2 explained 14·8% of variation with eigenvalue 1·336, and PC3 explained 13·4% of variation with eigenvalue 1·210).

Finally, we tested whether predator community composition together with biotope can cause varying selection on the artificial moths (Table 2). We assembled a model incorporating planned comparisons, where we included the main effects of the artificial moths' colour and melanization, the three predator community variables (PCs) and biotope as fixed factors (Table S6, Supporting information), as well as all two-way interactions for the artificial moths' colour and melanin pattern, and three-way interactions among colour, melanin and predator communities, and colour, melanin and habitat (Table S6, Supporting information).

Results

In total, 3710 artificial moths were used and 477 (12·9%) of those were attacked. Overall, yellow morphs were attacked less than white morphs (Table 1). The frequency of the wild *P. plantaginis* colour morphs in the local population did not affect the attack risk of yellow and white artificial moths (Fig. 2), as no main effect of natural frequency or interaction with attack risk was found (Table 1).

Table 2. The effect of predator community on attack risk of the colour morphs. The GLMM represents the best fit model to explain attack risk in relation to colour morph (colour, melanin) and predator community

Source	Estimate	SE	Z	P
(Intercept)[a]	−2·267	0·335	−6·753	**< 0·001**
Morph [yellow]	−0·368	0·141	−2·607	**0·009**
Melanin [more]	0·312	0·139	2·237	**0·025**
Predator community (PC1)	0·100	0·103	0·975	0·329
Predator community (PC2)	0·196	0·097	2·014	**0·044**
Predator community (PC3)	−0·146	0·148	−0·989	0·322
Biotope [forest]	−0·013	0·212	−0·065	0·948
Biotope [bush]	−0·504	0·274	−1·835	0·065
Predator community (PC3) *morph	0·381	0·147	2·579	**0·009**
Predator community (PC3) *melanin	−0·174	0·138	−1·256	0·209

Morph = colour morph (white or yellow) of artificial moth, melanin = hind wing melanization of artificial moth, PC1 = predator community principal component number one, PC2 = predator community principal component number two, PC3 = predator community principal component number three, biotope = biotope in which moths were displayed. The spatial effects of transects are nested within population and incorporated as random effects in the intercept. Significant P values are denoted in bold. [a]intercept includes factor levels: morph [white], melanin [less], biotope [open].

When we tested whether predator community compositions (PCs) together with habitat type affected morph attack risk, we found that relationship between attack rate and PC1 was not significant (Fig. 3a), whereas there was a significant effect of PC2 on overall attack rate (Table 2, Fig. 3b). Interestingly, there was a significant interaction between PC3 and colour morph on attack risk (Table 2, Fig. 3c). The yellow morph was attacked less than the white morph when predator community was characterized by Paridae (Fig. 3c). However, as the community shifted towards one characterized by Prunellidae, the yellow morph was attacked more often than the white (Fig. 3c). Larger area of melanization of hind wings increased attack risk, and moths tended to draw more attacks in bush habitats compared to open areas or forests (Table 2). All tested three-way interactions were non-significant.

Discussion

Here we use a field-based experiment which shows for the first time that predator community composition can explain the maintenance of polymorphism in warning coloration. According to our results, heterogeneity in predator community composition can generate a selection mosaic facilitating the evolution of polymorphic warning signals. Overall, predation was less frequent on the artificial prey resembling yellow male morph of *P. plantaginis* compared to the white morph, regardless of colour morph frequencies in the local, wild subpopulations. However, the direction of selection for the warning signal changed depending on the local predator community composition.

Conventional view maintains that the efficacy of a warning signal is positively frequency-dependent (Gamberale & Tullberg 1998; Lindström *et al.* 2001; Sherratt & Beatty 2003; Beatty, Beirinckx & Sherratt 2004). In this study, we did not find any evidence that predators would have selected against rare morphs: selection by predators favoured the more conspicuous yellow morph (Nokelainen *et al.* 2012) over the white morph irrespective of the locally dominant colour morph. This is in accordance with previous findings that the yellow morph is better protected against avian predators (Nokelainen *et al.* 2012; Hegna *et al.* 2013) and many other studies that have demonstrated a benefit of a more conspicuous signal in deterring predators (e.g. Lindström *et al.* 1999; Gamberale-Stille 2001; Merilaita & Tullberg 2005; Prudic, Skemp & Papaj 2007; Lindstedt, Lindström & Mappes 2008). Increase in melanization also resulted in higher predation risk. This is probably because the increase in black patterning reduced the area of yellow/white colour, making it

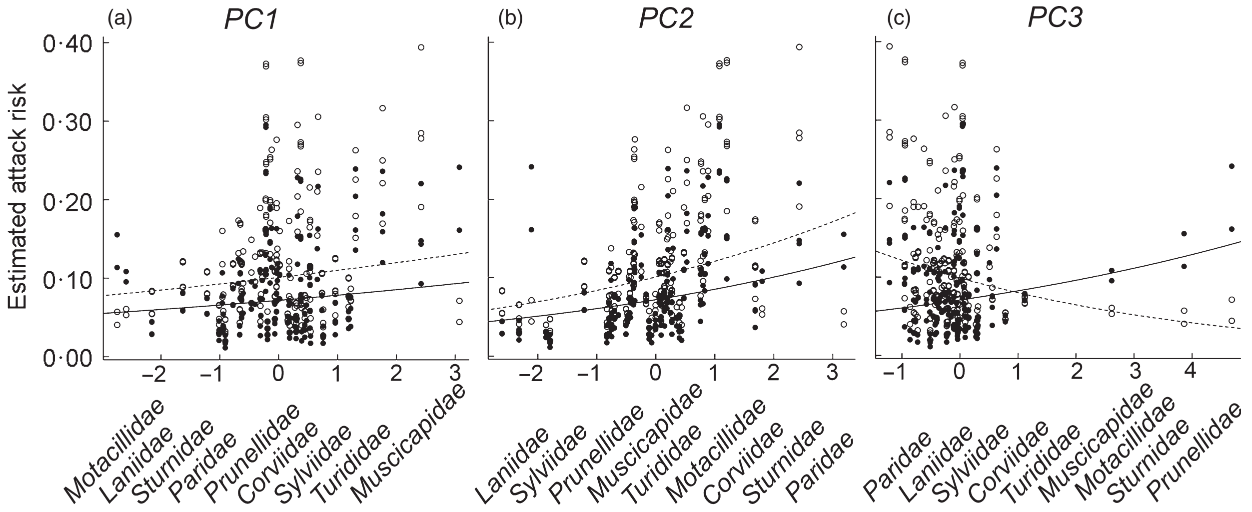

Fig. 3. The relationship between wing colour morph (white = open circles and dashed line, yellow = black circles and solid line) and predator community on predation risk. In all panels, *X*-axis represent principal component (PCs) analysis where factor scores represent bird communities of passerine families ordered according their factor loading values from the smallest to the largest (see Table S5, Supporting information). *Y*-axis represent predicted attack risks based on GLMM. There is no relationship between the first component and the attack risk (a). PC2 has significant effect on overall attack risk (b). There is an interaction effect of PC3 and colour morph on attack risk. The yellow morph was attacked less than the white morph when predator community was characterized by Paridae but the yellow morph was attacked more often than the white when the predator community was characterized by Sturnidae and Prunellidae (c).

difficult for the predators to recognize the hind wing pattern as a warning signal (Bohlin, Tullberg & Merilaita 2008; Hegna *et al.* 2013). The reason why we did not detect frequency-dependent predation on *P. plantaginis* colour morphs could be that the effect is overridden by other factors affecting selective regimes (i.e. locally varying predator community, see below). It is also possible that bird predators do not learn the warning signal of *P. plantaginis* per se, but instead generalize their avoidance (or preference) from earlier experience with other prey species (Alatalo & Mappes 1996; Gamberale & Tullberg 1996; Darst & Cummings 2006; Ham *et al.* 2006; ten Cate & Rowe 2007; Ihalainen *et al.* 2012). Thus, if similar-looking, defended interspecifics are present in the prey community, they may reinforce the warning signal, but if signal bearers are sparse in relation to palatable alternative prey with similar appearance, birds can forget or be confused about the warning signal (Speed 2000; Sherratt & Beatty 2003).

The importance of predator community composition was highlighted by an interaction between colour morph and predator community, namely the dominance of Paridae or Prunellidae, which explained the attack risk of the artificial moths. The yellow warning signal was particularly advantageous when the predator community comprised of Paridae. As the community shifted towards one characterized by Prunellidae, the superiority of yellow (or inferiority of white) warning signal was reversed: white became the more efficient warning signal. Prunellidae typically feed near ground level (Davies & Lundberg 1984; Svensson, Mullarney & Zetterström 2009), and white could perhaps be more efficient warning signal than yellow in that light environment. We note, however, that the shift towards a community dominated by Prunellidae could also be interpreted as a change towards a community characterized by pipits, wagtails, thrushes, warblers, flycatchers and crows, and therefore, Prunellidae may not be the only agent reversing the benefits of the colours. Competition within the predator community has potentially an effect on what selection is like for the prey (Holt 1977; Abrams 2000). It is curious that in polymorphic locations, yellow morph seems to have an advantage, but in monomorphic populations, it does not. Understanding this requires further investigation. It, however, indicates that the lack of white morphs in Scotland and yellow morphs in Estonia cannot solely be explained by predation. Nevertheless, our results support the suggestion that variation in predator community composition can be important factor causing heterogeneity in selection pressures, which promotes polymorphism in aposematic species as the fitness of colour morphs varies locally (Mallet & Joron 1999; Thompson 1999, 2005; Endler & Mappes 2004; Valkonen *et al.* 2012).

The predation pressure varied considerably between study sites. High attack rates in Scotland are probably due to the higher visibility costs in open moor habitats. However, the bird predation is probably only one piece in the puzzle. It is possible that the abundance of alternative prey affects predator behaviour in respective sites and influences attack rates on aposematic moths (Kokko, Mappes & Lindström 2003; Lindström *et al.* 2004). It is important to acknowledge that selection can take both spatial and temporal trajectories. Spatial variation can render individual traits to different conditions that are favourable in other local conditions but not in others (Mallet & Joron 1999; Thompson 1999, 2005; Endler & Mappes 2004). On the other hand, selection can have temporal aspect as well. For example, some arctiids also have to deal with facing with diurnal (birds) but also nocturnal (bats) predators in multispecies predator communities (Ratcliffe & Nydam 2008), which can render individuals to differential predation, depending on the time window they use. Wood tiger moth, however, is considered mostly diurnal species.

To conclude, selection can operate on relatively small spatial scales generating a mosaic of selection pressures promoting colour polymorphism in aposematic species. This also suggests that similar processes are likely to affect the evolution of other, non-aposematic species that exhibit colour polymorphism. Altogether, this study helps to understand how predator–prey interactions maintain variation in adaptive coloration.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Calculated contrasts of moth colours against the standard green background.

Table S2. The functional bird groups.

Table S3. The observed bird groups (at family taxon level) in each geographical location.

Table S4. Sums of the observed bird species in each geographical location.

Table S5. Principal component factor loadings of the most relevant predator groups.

Table S6. GLMM selection for the effect of predator community composition on attack risk of the colour morphs.