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LETTER

Geographic mosaic of selection by avian predators on hindwing warning colour in a polymorphic aposematic moth

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Abstract

Warning signals are predicted to develop signal monomorphism via positive frequency-dependent selection (+FDS) albeit many aposematic systems exhibit signal polymorphism. To understand this mismatch, we conducted a large-scale predation experiment in four countries, among which the frequencies of hindwing warning coloration of the aposematic moth, *Arctia plantaginis,* differ. Here we show that selection by avian predators on warning colour is predicted by local morph frequency and predator community composition. We found +FDS to be the strongest in monomorphic Scotland and lowest in polymorphic Finland, where the attack risk of moth morphs depended on the local avian community. +FDS was also found where the predator community was the least diverse (Georgia), whereas in the most diverse avian community (Estonia), hardly any models were attacked. Our results support the idea that spatial variation in predator communities alters the strength or direction of selection on warning signals, thus facilitating a geographic mosaic of selection.

Keywords

Aposematism, *Arctia plantaginis*, colour polymorphism, frequency-dependent selection, predator–prey interactions, predators, signal convergence, signal variation, wood tiger moth.

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INTRODUCTION

The survival strategy of aposematism, wherein prey use warning signals that predators learn to associate with their unprofitability and subsequently avoid, has stimulated biological studies for centuries (Wallace, 1867; Poulton, 1890; Cott, 1940; Mappes *et al*., 2005; Merrill *et al*., 2015; Skelhorn *et al*., 2016; Ruxton *et al*., 2018). In aposematism, prey benefit from lowered costs of predator education by carrying a common signal, whereas predators reduce risks by not attacking defended prey. This results in selection for local similarity in warning signals, a view that has been corroborated by theoretical approaches (e.g. Müller, 1878; Mallet and Joron, 1999; Sherratt, 2008; Aubier and Sherratt, 2015), laboratory experiments (e.g. Greenwood *et al*., 1989; Lindström *et al*., 2001; Rowland *et al*., 2007) and field studies (e.g. Mallet and Barton, 1989; Kapan, 2001; Borer *et al*., 2010; Dell'aglio *et al*., 2016; Chouteau *et al*., 2016). Nevertheless, phenotypic variation and polymorphism in aposematic organisms are widespread in nature (e.g. frogs: Rojas, 2017; Siddiqi *et al*., 2004; newts: Beukema *et al*., 2016; Mochida, 2011; butterflies: Merrill *et al*., 2015; moths: Brakefield and Liebert, 1985; bumblebees: Plowright and Owen, 1980; beetles: Bocek and Bocak, 2016; Brakefield, 1985; locusts: Nabours, 1929; myriapods: Marek and Bond, 2009; nudibranchs: Winters *et al*., 2017), which requires an evolutionary explanation.

Given that the association between prey warning signal and defence should be learned by each generation of predators (Mappes *et al*., 2014), the benefit of signal sharing depends on how often predators encounter the signal. The encounter rate then depends on both the frequency (Müller, 1879; Heino *et al*., 1998) and density (Müller, 1879; Sword, 1999; Rowland *et al*., 2007; Endler and Rojas, 2009) of prey carrying the signal. Thus, it is expected that selection on aposematism is positively frequency-dependent (+FDS), with predators avoiding the most common warning signal in a locality (Sherratt, 2008; Comeault and Noonan, 2011; Chouteau and Angers, 2011; Chouteau *et al*., 2016; Ruxton *et al*., 2018).

On the other hand, several mechanisms have been proposed to counterbalance selection for signal monomorphism and facilitate warning colour polymorphism (reviewed in Briolat *et al*., 2018). For example temporally and spatially varying interspecific interactions can result in geographically variable patterns of polymorphism (McLean & Stuart-Fox 2014), particularly when coupled with limited amounts of gene flow between differentially selected populations (e.g. Merilaita, 2001; Gordon *et al*., 2015; Aubier and Sherratt, 2015). Often these mechanisms are thought to act simultaneously, or alternate in time or space (Mallet and Joron, 1999; Gray and McKinnon, 2007; Stevens and Ruxton, 2012) creating a geographic mosaic of selection (Thompson, 2005). Although both theoretical (e.g. Gordon *et al*., 2015; Aubier and Sherratt, 2015; Holmes *et al*., 2017) and experimental work (e.g.

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Willink *et al*., 2014; Aluthwattha *et al*., 2017) have identified several mechanisms that allow multiple morphs to persist, there is no conclusive evidence from the field and the relative importance of different selective agents is not well understood (Stevens and Ruxton, 2012; Chouteau *et al*., 2016). Alas, there is little empirical evidence as to the role of predator communities on local or global morph frequencies of aposematic prey.

The variation in the degree of warning colour polymorphism shown by the wood tiger moth (*Arctia plantaginis*) across the Western Palaearctic provides an excellent system to study how warning signal variation is maintained in the wild (Hegna *et al*., 2015). At a local scale, predator community structure (Nokelainen *et al*., 2014) and sexual selection (Nokelainen *et al*., 2012; Gordon *et al*., 2015) have been found to alter the direction of selection on white and yellow male morphs, but no previous studies have addressed selection on a wide geographical scale and including *A. plantaginis* females, which are red or yellow. We exposed artificial moths representing the three hindwing colour morphs (white, yellow, red), to local predators in a field experiment spanning across four countries, while monitoring the abundance and community structure of local predator species. We tested whether (1) selection by predators favours the locally common morph; (2) the community structure of avian predators is associated with the predation pressure on different morphs; and (3) there is variation in the direction or strength of selection among populations, matching the local morph frequencies. Variable selection pressure is one of the main candidate mechanisms for the maintenance of polymorphism. By our work, we provide the best-documented case to date of a geographic mosaic of selection on warning signals at broad spatial scales.

MATERIAL AND METHODS

Study system

Adult wood tiger moths, *Arctia plantaginis* (Erebidae: Arctiinae; formerly *Parasemia*; see Rönkä *et al*., 2016 for classification), show conspicuous warning colours and possess a chemical defence fluid, which contains pyrazines (Rojas *et al*., 2017; Burdfield-Steel *et al*., 2018b) and is a deterrent to avian predators (Rojas *et al*., 2017; Burdfield-Steel *et al*., 2018a). Their warning coloration varies throughout their Holarctic distribution, but local polymorphism is common too (Hegna *et al*., 2015). In the Western Palaearctic male hindwing colour is either white or yellow, or varies more continuously between yellow and red as seen in females. We selected four study locations that represent the colour variation continuum from monomorphic to polymorphic *Arctia plantaginis* populations in the Western Palaearctic (Fig. 1). For the purposes of this study, we assigned both sexes to belong to the white, yellow or red morph based on their hindwing colour, and simplify the continuous hindwing coloration of females and Georgian males into two classes: yellow and red (categorised by human eye in 6 grades as in Lindstedt *et al*., 2011), here grades 1-2 are determined yellow and 3-6 red). Accordingly, Scotland is monomorphic with yellow males and females, Georgia is mostly red with 4.3% of males being yellow and Estonia and Finland are polymorphic with all females caught between 2013 and 2015 classified as red, and males as either white or yellow (Fig. 1).

Wood tiger moths are widespread but often low in numbers. Therefore, colour morph frequencies were calculated by population based on annual surveys using pheromone traps and netting between 2009 and 2014 in Scotland, and 2013–2015 in Georgia, Estonia and Finland. Morph frequencies for white and yellow males, and yellow or red males in Georgia, were calculated as the average frequencies from all data available. Morph frequencies for yellow and red females were based on netting data, as the pheromone traps only lure male moths. Because our data set was thus biased towards male moths, we corrected the morph frequencies according to a sex ratio of 45 females to 156 males, based on a mark–release–recapture study spanning two years in Central Finland (Gordon *et al*. unpublished data). This sex ratio was used, as it is likely to depict the detectability of each morph more accurately than an even 1:1 sex ratio. The higher frequency of males to females is supported by two observations: male wood tiger moths live longer and fly more actively than females, and the adult sex ratio immediately after eclosion is slightly biased towards males even in laboratory conditions (K. Suisto, personal communication). The concluding morph frequencies (Fig. 1a and 2a) are consistent with museum samples (Hegna *et al*., 2015) and laboratory stocks originating from the four study populations (Central-Finland, Estonia, Scotland and Georgia).

Predation experiment

To estimate the attack risk of white, yellow and red hindwing colour morphs by local predators in the wild we used artificial moth models, resembling real *A. plantaginis* morphs. Models with plasticine (Caran D'Ache Modela 0259.009 Black) bodies attached to printed waterproof (Rite in the Rain ©, JL Darling Corporation, Tacoma, WA, USA) paper wings were prepared following methods described in Nokelainen *et al*. (2014). Models were constructed using pictures of one white moth hindwing and two forewings, one with a typical European pattern and another with a typical Caucasian (Georgian) pattern, which were copied and assembled in GIMP 2.8.16 SOFTWARE (GNU Image manipulation program) to create six models representing the white, yellow and red morphs in Europe and Georgia (Fig. 1b). A locally common forewing type was used to reduce potential novelty effect caused by the forewing pattern (Hegna and Mappes, 2014). Resemblance of the artificial models to the real moths was verified by taking measurements of reflectance from the black and coloured areas of real moth wings and printed wings with a Maya2000 Pro spectrometer (Ocean Optics) using a PX-2 Pulsed Xenon Light Source (Ocean Optics) for illumination and adjusting the model colours with Gimp (2.8.16) to match the natural wing colour as closely as possible with a calibrated (HP Colour LaserJet CP2025) printer (spectral match between printed moths and real wings was inspected by visual comparison of reflectance curves as in Rönkä *et al*., 2018, where identical models were used, and a detailed avian vision model with JNDs' of all three morphs on different backgrounds is reported in Henze *et al*., 2018). Thus, we can expect all avian

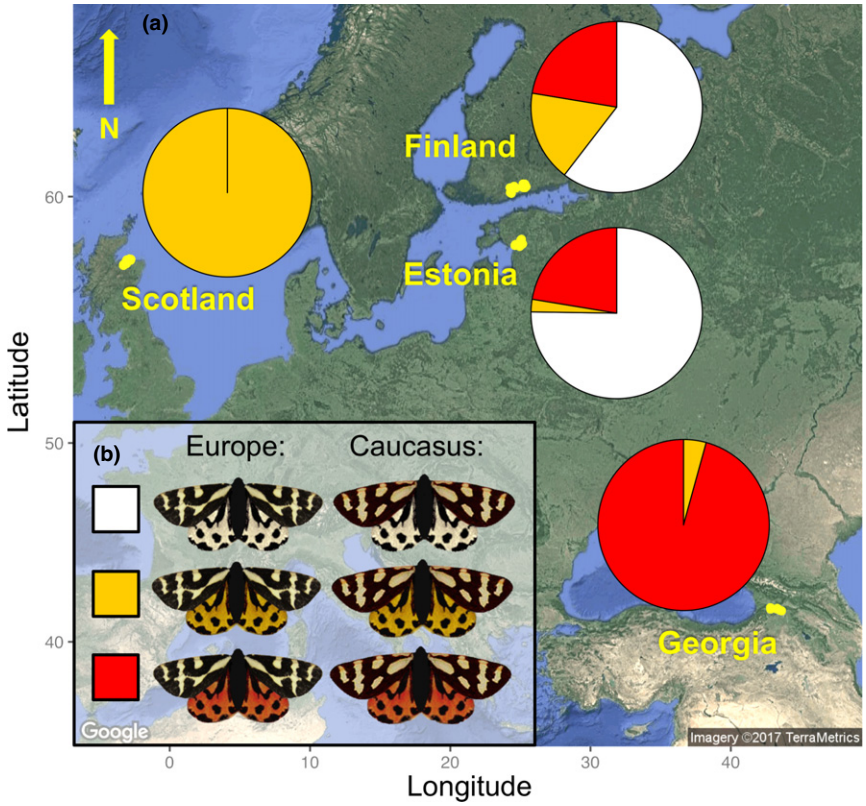

Figure 1 (a) Study populations and local morph frequencies and (b) moth models used in Europe and Georgia representing a local forewing pattern and white, yellow or red hindwing morph. At the monomorphic end of the monomorphic-polymorphic continuum in Scotland, both sexes have yellow hindwing coloration. In Georgia red is the dominating hindwing colour, but male coloration varies continuously towards yellow. In Estonia, white hindwing colour dominates, as the males are almost exclusively white and females red. In Southern Finland all three colour morphs are present (white and yellow male morphs and females vary continuously from yellow to red).

predators to see the moth dummies similarly as they would see the real moths, regardless of birds' visual properties, which may vary among species. As our study focused on the hindwing coloration, all other variables such as wing size and pattern were kept constant.

We set up 60 predation transects across the four study populations (15 in each country) in open, semi-open and closed natural habitats where the wood tiger moth and its potential avian predators were known or presumed to occur. The predation transects were set at least 500 m apart to avoid birds having overlapping territories between the transects. Along each 900 m transect 20 white, 20 yellow and 20 red artificial moth models were set individually every 15 meters using a randomised block design, so that two models of the same colour would never be next to each other. Models were pinned directly on natural vegetation, either to green leaves large enough to support their weight, or to tree trunks, as visibly as possible. All models were left in the field for a maximum of 6 days (2–6 days, 4 days on average), during the *A. plantaginis* flight season in 2014 (May 31st – July 6th in Estonia, May 26th–July 6th in Finland, June 15th–July 30th in Scotland and July 12th–August 3rd in Georgia). Predation events were recorded every 24 hours except for days of heavy rain (as birds were likely not active). For practical reasons (i.e. accessibility of mountain roads and weather conditions) the protocol was modified in Georgia. The 20 white, 20 yellow and 20 red models were set every 10 m totalling up to 600 m, left in the field for three consecutive days (72 h), and checked only once.

Attacks were recorded based on imprints on the plasticine body and fractures in the wings (see Supplemental Experimental Procedures). Only clear avian attacks were included in the analyses (Tables S1 and S2). Missing and attacked models were replaced with a new model of the same colour to ensure constant morph frequency during the experiment. Excluding or keeping consecutive attacks on the replaced models in the analyses did not markedly change the outcome, reported here (Table 1) for the data set including replaced models (4004 observations) and for the data set including original models only (3600 observations; in Supplemental Table S3). Therefore, we kept the replaced models in for all of the analyses, as it increased the sample size.

Measures of predator community

To estimate the abundances of different insect-feeding birds, which are the most likely predators of wood tiger moths, we counted birds belonging to the orders Passeriformes and Piciformes (Supplemental Table S3). These counts were done once, either before or during the predation experiment, along

Table 1 Positive frequency-dependency of the estimated attack risk

(a) Model selection	Δ d.f.	LRT	Pr(Chi)	model AIC
colour * morph frequency				3957.0
colour + morph frequency	2	0.1949	0.907	3953.2

(b) Model 1a Random effects	Variance	SD
transect within country	0.3315	0.5758
country	0.1633	0.4041

Fixed effects	Estimate	SE	*Z*-value	*P*-value
(Intercept): colour[w]	−3.0433	0.2275	−13.376	**< 0.001**
colour[y]	−0.0923	0.0940	−0.982	0.3259
colour[r]	−0.0841	0.0925	−0.909	0.3633
morph frequency	−0.3728	0.1071	−3.481	**0.0005**

The asterisk (*) denotes both main effects and interaction terms used.
(a) The model including only main effects of morph frequency and morph colour (underlined) was selected because we did not find a significant interaction. (b) Estimates of Model 1a. Values of significance level < 0.05 are bolded. Δ d.f. denotes change in model degrees of freedom.

the predation lines using a modified transect count method (see Nokelainen *et al*., 2014). Bird species observed only in one transect (out of 60), or clearly not adapted to prey on moths (e.g. crossbills), were excluded from analysis. Observations were done within 25 m from the middle of the transect in calm weather between 6 am and 1 pm, when birds were most active. Shannon–Wiener diversity index (Fig. 3) was calculated using R package 'vegan' 2.5-6 (Oksanen et al. 2013).

Statistical analyses

To investigate how local predator community affects the direction and strength of selection on wood tiger moth morphs, we constructed generalised linear mixed models. Because the artificial moths were presented to predators over a different number of days in each transect, the attack risk (attacked or not) within a day exposed was used as the response variable for all analyses, modelled with a binomial distribution and a logit link function. First, we tested whether predators select for wood tiger moth warning colours in a frequency-dependent manner across populations (Fig. 2). For this, we used local morph frequency calculated from field monitoring data and its interaction with colour morph as the explanatory variables in Model 1 (Table 1). Transect ID, nested within country, was set as the random factor to account for the nested spatial structure of the study design.

To test for predator community composition effects, the dimensions of the bird count data, consisting of 12 genera, was first reduced with a principal component analysis using the R function 'princomp'. To avoid overparameterisation, the main effects of the first three resulting components (explaining 44.7 %, 33.7 % and 8.5 % of the variation in predator community), and their three-way interactions with morph colour and country, were included one by one as explanatory variables in three separate GLMMs (Table 2). Country was included as an explanatory variable to test for local differences in selection and thus transect ID alone was set as a random effect to each model.

Statistical models were simplified using a backward stepwise deletion method based on significance of terms in the models. Variables were excluded one by one from the full models, until only main effects or significant interactions were left in each model. All analyses were performed with R (RCoreTeam, 2013) in RStudio 0.99.491 (RStudio Team, 2015), using the package *lme4* (Bates et al. 2015).

RESULTS

Positive frequency-dependent selection

Altogether, we observed a total of 718 bird attacks on the 4004 artificial moths (Table S1). The relative attack risk of each colour morph was lower when the natural frequencies of the respective morph were higher in relation to the others (Table 1, Fig. 2). Also, the morphs with intermediate local frequencies show corresponding levels of attack risk (Fig. 2). This effect did not depend on colour morph itself (Table 1, Table S2), as expected if the local predator avoidance depends more on local morph frequency than on morph colour.

Predator community

The attacks were not evenly distributed across countries or transects (Fig. 2c). Predation pressure varied between and within countries, being highest in Scotland and lowest in Estonia (Fig. 2c). Georgia had the lowest amount of insect feeding birds observed (2.1 per 100 meters) compared to Finland (2.6), Scotland (4.0) and Estonia (4.4) respectively. Georgia also had the least diverse predator community measured with Shannon–Wiener diversity index, whereas Estonia was most diverse, followed by Scotland (Fig. 3). Across countries, the three most commonly observed potential predators included the common chaffinch, the willow warbler (replaced by green warbler in Georgia) and the great tit (Supplemental Table S4), the latter of which was observed to attack the artificial moths. The first three principal components (PC1, PC2 and PC3) that explained 44.7 %, 33.7 % and 8.5 %, respectively, captured 87.0 % of variance in the predator community data. PC1 was dominated by Sylvidae (warblers), Fringillidae (finches) and Muscicapidae (flycatchers), which loaded in the negative end, whereas the positive end of the axis was loaded with Paridae (tits) (Fig. 3). PC2 was dominated by Paridae and PC3 with Fringillidae, Muscicapidae and Troglodytidae (the Eurasian wren) (see Supplemental Table S5 for factor loadings).

Significant association between predator community structure and selection

A consecutive analysis, where the effect of predator community on the attack risk of each moth colour morph was addressed, revealed a significant three-way-interaction between moth colour, country and PC1 (Model 2, Table 2a, Fig. 3). This significant interaction means that the variation in predator community structure captured by PC1 is associated with predation pressure on different colour morphs, but the

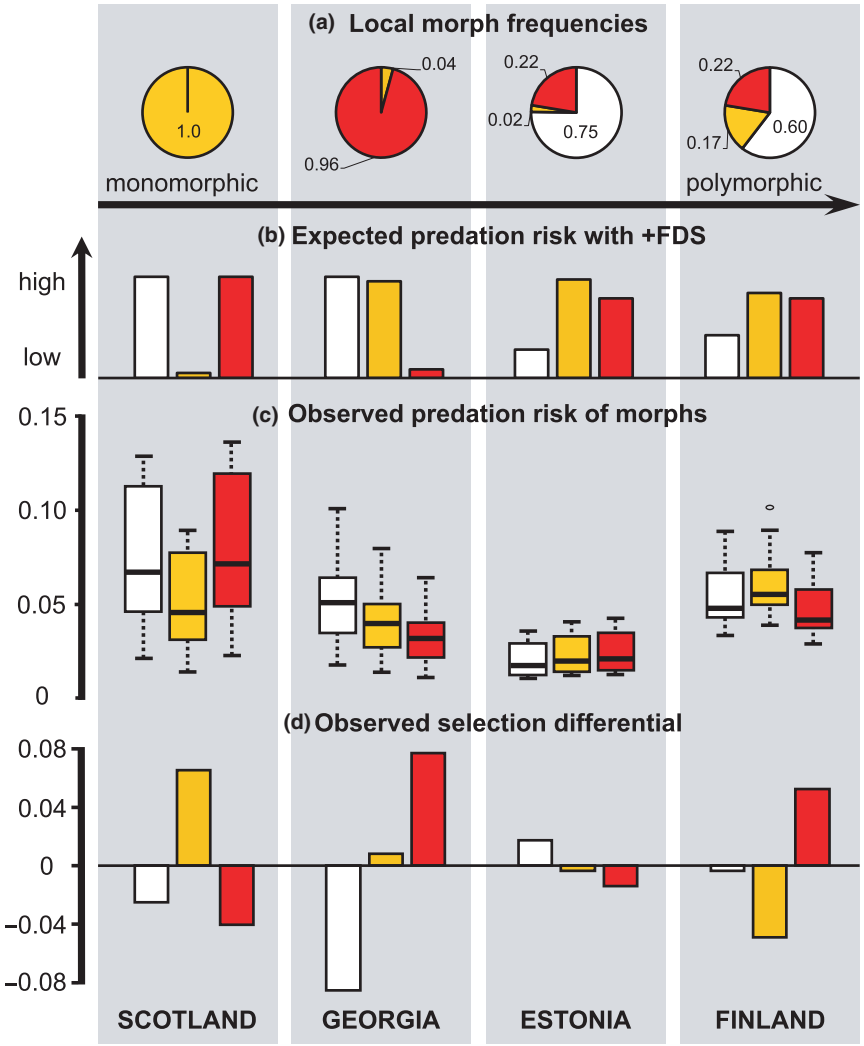

Figure 2 Wood tiger moth morph frequencies compared to expected and observed predation risk and selection differential by country. (a) Local morph frequencies calculated from annual monitoring data, (b) expected attack risk according to the + FDS hypothesis, where each morph is attacked according to its local frequency, (c) observed predation illustrated as GLMM estimates of daily attack risk for each morph by country and (d) observed difference in attacks per morph compared to a situation where all morphs would be attacked equally. Morph colours (white, yellow, red) as in Figure 1.

direction of the association is different between countries (i.e. between local communities). PC2 and PC3 were not significantly associated with predation pressure (Table 2c and 2d).

DISCUSSION

Our experiment is among the few experimental approaches integrating community-level interactions into the study of selection on warning signals (Mochida, 2011; Valkonen *et al.*, 2012; Nokelainen *et al.*, 2014; Aluthwattha *et al.*, 2017), and the first to do so on such a large geographical scale. With a wide-ranging field experiment spanning populations varying in their degree of polymorphism, we demonstrate that local bird predators avoid locally common morphs, but also that both the strength and direction of selection on warning colour varies geographically. We found that changes in local predator communities drive geographical variation in selection despite positive frequency-dependence. Local predator–prey interactions are thus contributing to the maintenance of both geographical variation and local polymorphism in warning signals.

Local avian predators appear as key in driving warning colour evolution, which can take different evolutionary trajectories over a geographical scale. Here, the effect of predator community on attack risk towards each morph varied significantly across countries. The first component from the principal component analysis, explaining 44.7 % of the variation in the abundances of insectivorous birds in different families, significantly affected estimated risk of attack. However, it did so differently towards each morph in the different countries. Interpreting the component loadings and model estimates (Table 2, Fig. 3), the Paridae (e.g. tits) and Prunellidae (consisting of only one species, the dunnock, *Prunella modularis*) selected for different morphs in different countries. Our results corroborate the predator community effects found by Nokelainen *et al.* (2014), as we also found that in Finland the

Table 2 The interaction effect of predator community and location (country, C) on the attack risk towards the wood tiger moth colour morphs

(a) Model selection with PC1	Δ d.f.	LRT	Pr (Chi)	model AIC
PC1*colour*C				3956.1
PC1 + colour+C + PC1:colour + PC1: C + colour:C	6	14.35	**0.026**	3958.4

(b) Model 2 Random effects	Variance	SD
Transect	0.2779	0.5272

Fixed effects	Estimate	SE	Z-value	*P*-value
(Intercept): colour[w], C[Finland]	−3.556	0.438	−8.126	**< 0.001**
colour[y]	0.869	0.407	2.134	**0.033**
colour[r]	0.370	0.440	0.840	0.401
PC1	0.139	0.088	1.590	0.112
C[Estonia]	−0.309	0.621	−0.498	0.619
C[Georgia]	0.618	0.647	0.955	0.340
C[Scotland]	0.843	0.477	1.766	0.077
PC1: colour[y]	−0.164	0.083	−1.989	**0.047**
PC1: colour[r]	−0.115	0.089	−1.299	0.194
C[Estonia]: colour[y]	−0.463	0.606	−0.765	0.444
C[Estonia]: colour[r]	0.297	0.618	0.481	0.631
C[Georgia]: colour[y]	−0.773	0.592	−1.306	0.192
C[Georgia]: colour[r]	−0.041	0.616	−0.067	0.947
C[Scotland]: colour[y]	−1.169	0.445	−2.624	**0.009**
C[Scotland]: colour[r]	−0.345	0.472	−0.730	0.466
PC1: C[Estonia]	−0.123	0.097	−1.273	0.203
PC1: C[Georgia]	−0.143	0.111	−1.280	0.201
PC1: C[Scotland]	−0.159	0.103	−0.551	0.121
C[Estonia]: PC1: colour[y]	0.197	0.093	2.115	**0.035**
C[Estonia]: PC1: colour[r]	0.176	0.099	1.781	0.075
C[Georgia]: PC1: colour[y]	0.105	0.105	0.991	0.322
C[Georgia]: PC1: colour[r]	−0.033	0.113	−0.294	0.769
C[Scotland]: PC1: colour[y]	0.256	0.100	2.550	**0.011**
C[Scotland]: PC1: colour[r]	0.091	0.102	0.892	0.372

(c) Model selection with PC2	Δ d.f.	LRT	Pr (Chi)	Model AIC
PC2*colour*country				3962.6
PC2 + colour+C + PC2:colour + PC2: C + colour:C	6	4.413	0.621	3955.0
PC2 + colour+C + PC2:colour + colour: C	3	3.334	0.343	3952.4
PC2 + colour+C + colour:C	2	3.923	0.141	3952.3
PC2 + colour+C	6	16.737	**0.010**	3957.0
Colour*country	1	0.545	0.460	3950.8

(d) Model selection with PC3	Δ d.f.	LRT	Pr (Chi)	Model AIC
PC3*colour*country				3963.6
PC3 + colour+C + PC3:colour + PC3: C + colour:C	6	5.190	0.520	3956.8
PC3 + colour+C + PC3:colour + colour: C	3	1.400	0.706	3952.2
PC3 + colour+C + colour:C	2	4.599	0.100	3952.8
PC3 + colour+C	6	16.726	**0.010**	3957.5
Colour*country	1	0.034	0.854	3950.8

(a) Model selection starting from the main effects, interactions and a three-way interaction between principal component 1 (PC1), country (C) and colour morph (colour) as the explanatory variables, with the best-fit model underlined. (b) Estimates from the selected model (Model 2) with a significant three-way interaction of principal component 1, colour morph and country. (c) Model selection for principal component 2. Principal component 2 had no significant effects on attack risk, and thus estimates are not shown. (d) Model selection for principal component 3. Principal component 3 had no significant effects on attack risk, and thus estimates are not shown. Values of significance level < 0.05 are bolded. Δ d.f. denotes change in model degrees of freedom.
The asterisk (*) denotes both main effects and interaction terms used.

(continues)

yellow morph is better protected in communities characterised by Paridae, whereas the white morph is favoured in communities characterised by Prunellidae. In contrast, an opposite effect was found in Scotland where the yellow morph dominates, suggesting that local predators can select for different colours depending on the communities they are exposed to.

Our experiment showed that across countries locally dominating colour morphs were attacked least, as predicted by + FDS. Thus, warning signal efficacy is enhanced with increasing frequency of similarly signalling individuals as predicted due to the number-dependence of predator learning and memorisation. Nonetheless, we found geographical variation in the strength of predator-induced selection. Comparison with previous experiments in those study areas that overlap (Nokelainen *et al*., 2014) also reveal temporal differences. We found high overall predation pressure in Scotland where the yellow morph was in favour compared to other study locations. Although Nokelainen *et al*. (2014) did not detect positive frequency dependency, they also found much higher overall attack rates in Scotland compared to Southern Finland and Estonia. On the other hand, Nokelainen *et al*. (2014) found that yellow males were significantly less attacked than white males in Southern Finland, whereas in our study the yellow morph tended to have more attacks than the other morphs. Interestingly, the frequency of yellow and white morphs varies in Southern Finland in a biannual cycle (Galarza *et al*., 2014), and the yellow morph was more common during Nokelainen *et al*.'s (2014) study, whereas in contrast the white was more common during our experiment, suggesting again that the locally most common morphs have an advantage. Temporal fluctuations in local predator–prey interactions could therefore plausibly explain why estimates of predation pressure on different colour morphs conducted in different years have varied.

All morphs were attacked at equally low levels in Estonia, which implies spatial variation in the strength of selection or even locally relaxed natural selection on the warning signal. The low predation pressure is not explained by a low number of predators, as there were more insectivorous birds in Estonia than in any other study site (Table S4). The bird community composition in Estonia differed from the other countries though, being most diverse and characterised by Sylvidae, Fringillidae, Muscicapidae, Turdidae, Troglodytidae and Oriolidae, suggesting that the strength of selection was lower in

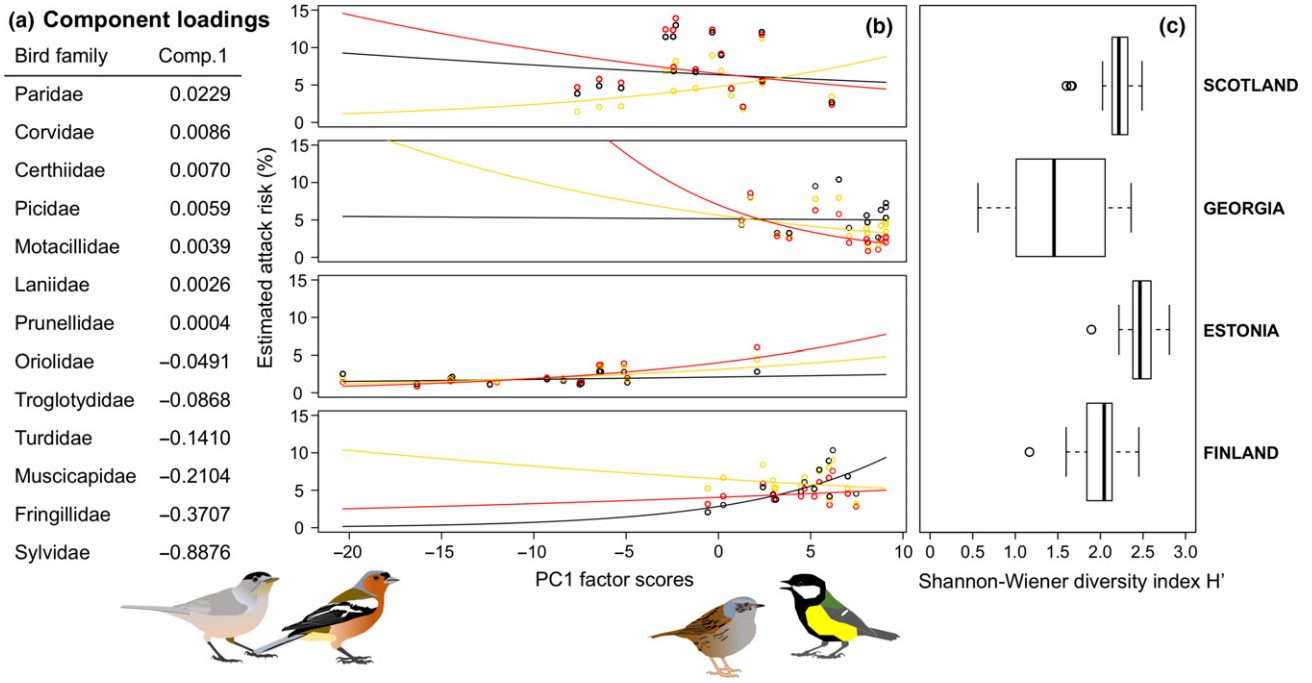

Figure 3 Community composition and diversity of insectivorous birds per population. Factor scores and loading of the first principal component describing 44.7% of the total variation in bird communities across countries. Panel (a) family level component loadings, panel (b) the three-way interaction effect of predator community on estimated attack risk of different colour morphs (black line corresponds to the white morph) at each transect illustrated by population, panel (c) Shannon–Wiener diversity indexes calculated per transect and plotted by population.

diverse communities as opposed to when Paridae (e.g. tits) characterised the community.

Other properties of the predator community that can affect the strength of selection on warning signals include the relative abundance of naïve vs. experienced predators (Mappes *et al.*, 2014), predators' capacity to learn many different signals (Beatty *et al.*, 2004), broad generalisation between the morphs (Balogh and Leimar, 2005; Sherratt, 2008), conflicting selection by different predators (Valkonen *et al.*, 2012; Nokelainen *et al.*, 2014), and the spatial arrangement of predators in relation to prey (Endler and Rojas, 2009). It is also possible that moth behaviour (e.g. flight activity) varies between habitats, sexes or colour morphs, leading to differences in exposure to potential predators (see e.g. Rojas *et al.*, 2015). While long distance mate attraction is achieved through female pheromone signalling, there is evidence of differential mating success between the white and yellow male morphs in Finland (Nokelainen *et al.*, 2012). Thus, sexual selection may well play a role in maintaining polymorphism in the Finnish population (Gordon *et al.*, 2015), but its role in other populations remains unknown.

In temperate regions, most insectivorous birds are migratory and prey population sizes are highly variable due to interseasonal weather variability. This is likely to cause variation in the relative abundances of naïve predators across the breeding season. Furthermore, local seasonal communities are continuously changing, altering the direction and/or strength of selection on warning signals (Mappes *et al.*, 2014). Siepielski *et al.* (2013) reviewed directional selection on phenotypes, and found that selection tends to vary more in strength than in direction between populations, with the majority of their examples coming from mid-latitudes in the northern hemisphere. Most experimental evidence of + FDS in the wild, however, comes from tropical systems (Mallet and Barton, 1989; Comeault and Noonan, 2011; Chouteau and Angers, 2011), where the prey and predator community composition is temporally less variable (Mittelbach *et al.*, 2007). In such communities, strong + FDS can lead to very accurate mimicry between warning coloured prey, whereas in more variable conditions, higher levels of variation and polymorphism can be maintained.

The paradoxical maintenance of local polymorphism despite + FDS could thus be explained by spatial and temporal variation in morph survival combined with individuals migrating between the subpopulations (Gordon *et al.*, 2015; McLean & Stuart-Fox 2014; Joron *et al.*, 1999). Differences in the level of population isolation, and thus gene flow between them, could explain part of the geographical variation in wood tiger moth warning colours. Population genetic evidence supports this theory: the red-dominated Georgian subspecies *A. p. caucasica* occurring in the mountains of Caucasus is genetically differentiated from all other sampled European populations, having a distinctive genomic composition from the rest of Western Palaearctic samples (Hegna *et al.*, 2015; Rönkä *et al.*, 2016; Yen *et al.*, 2020). The Finnish and Estonian populations clustered together away from the Scottish population, as would be predicted by effects of isolation by distance (Yen *et al.*, 2020). Thus, predator selection against rarity and genetic isolation of Georgian and Scottish populations can explain the monomorphism in those populations. Furthermore, the long-term co-existence of multiple morphs and the low genetic differentiation among

polymorphic populations in Finland with yearly variation in genetic structure (Galarza *et al*., 2014) indicate a role for gene flow along with varying predation pressure in maintaining local populations at different frequencies. Despite the genetic similarity and gene flow between Estonian and Finnish populations, Estonian moth populations are practically monomorphic white, whereas Finnish populations are polymorphic. Although the populations are connected, bird communities are remarkably different between them. Thus, we suggest that relaxed predator selection in Estonia together with sexual selection that seems to favour white morphs, especially when common, (see Nokelainen *et al*., 2012; Gordon *et al*., 2015) may explain the white dominance of this population.

As recently noted by several authors (e.g. Nokelainen *et al*., 2014; Skelhorn *et al*., 2016; Chouteau *et al*., 2016), more experimental work is needed to clarify predator–prey interactions at the community level in order to understand how selection is driving the evolution of warning signals in diverse natural ecosystems. Our experiment is so far the most comprehensive analysis showing how spatio-temporal variation in predator–prey communities affects the maintenance of within-species variation and evolutionary pathways to biodiversity. It shows that, while +FDS is acting in most populations, spatial variation in predator and prey communities alters the strength or direction of selection on warning signals, thus facilitating a geographical mosaic of selection which can maintain polymorphism.

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AUTHORSHIP

JM, KR, ON, JV, BR and SG designed the experiment. All authors contributed to the fieldwork. KR, JV and ON did the statistical analysis. KR led the writing and all authors contributed to it and accepted the final version.

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