

**This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.**

**Author(s):** Rönkä, Katja; De Pasqual, Chiara; Mappes, Johanna; Gordon, Swanne; Rojas Zuluaga, Bibiana

**Title:** Colour alone matters : no predator generalization among morphs of an aposematic moth

**Year:** 2018

**Version:** Accepted version (Final draft)

**Copyright:** © 2017 The Association for the Study of Animal Behaviour.

**Rights:** CC BY-NC-ND 4.0

**Rights url:** <https://creativecommons.org/licenses/by-nc-nd/4.0/>

**Please cite the original version:**

Rönkä, K., De Pasqual, C., Mappes, J., Gordon, S., & Rojas Zuluaga, B. (2018). Colour alone matters : no predator generalization among morphs of an aposematic moth. *Animal Behaviour*, 135(January), 153-163. <https://doi.org/10.1016/j.anbehav.2017.11.015>

1 **Colour alone matters: no predator generalization among morphs of an aposematic moth**

2 Katja Rönkä\*, Chiara De Pasqual\*, Johanna Mappes, Swanne Gordon & Bibiana Rojas

3 Department of Biological and Environmental Science, University of Jyväskylä, Centre of

4 Excellence in Biological Interactions, Finland

5 Received 12 June 2017

6 Initial acceptance 24 July 2017

7 Final acceptance 19 October 2017

8 MS number 17-00475R

9 \*Shared first authorship

10 Correspondence: K. Rönkä, Department of Biological and Environmental Science, University of  
11 Jyväskylä, Centre of Excellence in Biological Interactions, P.O. Box 35, FI-40014, University of  
12 Jyväskylä, Finland.

13 E-mail address: [katja.h.ronka@jyu.fi](mailto:katja.h.ronka@jyu.fi)

14

15 Local warning colour polymorphism, frequently observed in aposematic organisms, is  
16 evolutionarily puzzling. This is because variation in aposematic signals is expected to be selected  
17 against due to predators' difficulties associating several signals with a given unprofitable prey. One  
18 possible explanation for the existence of such variation is predator generalization, which occurs  
19 when predators learn to avoid one form and consequently avoid other sufficiently similar forms,  
20 relaxing selection for monomorphic signals. We tested this hypothesis by exposing the three  
21 different colour morphs of the aposematic wood tiger moth, *Arctia plantaginis*, existing in Finland  
22 to local wild-caught predators (blue tits, *Cyanistes caeruleus*). We designed artificial moths that  
23 varied only in their hindwing coloration (white, yellow and red) keeping other traits (e.g. wing  
24 pattern and size) constant. Thus, if the birds transferred their aversion of one morph to the other two  
25 we could infer that their visual appearances are sufficiently similar for predator generalization to

26 take place. We found that, surprisingly, birds showed no preference or aversion for any of the three  
27 morphs presented. During the avoidance learning trials, birds learned to avoid the red morph  
28 considerably faster than the white or yellow morphs, confirming previous findings on the efficacy  
29 of red as a warning signal that facilitates predator learning. Birds did not generalize their learned  
30 avoidance of one colour morph to the other two morphs, suggesting that they pay more attention to  
31 conspicuous wing coloration than other traits. Our results are in accordance with previous findings  
32 that coloration plays a key role during avoidance learning and generalization, which has important  
33 implications for the evolution of mimicry. We conclude that, in the case of wood tiger moths,  
34 predator generalization is unlikely to explain the unexpected coexistence of different morphs.

35

36 **Keywords.**

37 learning, polymorphism, predator generalization, predator–prey interactions, warning signals, wood  
38 tiger moth

39

40 Aposematic organisms display warning signals that predators learn to associate with their  
41 unprofitability (Poulton, 1890). The survival of such prey is thus highly dependent on a predator's  
42 ability to learn, remember and generalize their learned avoidance to other individuals sharing the  
43 same warning signal (reviewed in Ruxton, Sherratt, & Speed, 2004). Signal sharing among  
44 aposematic prey benefits both the prey and their potential predators: (1) a given individual has a  
45 lower risk of predation when more individuals share the same warning signal, and (2) predators  
46 benefit from not having to sample as many unprofitable or toxic prey and can more easily  
47 remember one and not multiple signals (Ghirlanda & Enquist, 2003; Guilford & Dawkins, 1991;  
48 Müller, 1878; Rowland, Ihalainen, Lindström, Mappes, & Speed, 2007; ten Cate & Rowe, 2007).  
49 Therefore, local polymorphism in warning coloration is expected to be selected against (Chouteau,

50 Arias & Joron 2016; Endler 1991; Joron & Mallet, 1998; Lindström, Alatalo, Lyttinen, & Mappes,  
51 2001; Mallet & Barton, 1989; Mallet & Joron, 1999; but see also Ihalainen, Lindström, & Mappes,  
52 2007 who found no evidence for slower avoidance learning of single versus multiple signals).

53         Despite the predicted disadvantages, warning signal polymorphisms are present in several  
54 aposematic taxa, such as frogs (Amézquita, Castro, Arias, González, & Esquivel, 2013; Rojas &  
55 Endler, 2013), ladybirds (O'Donald & Majerus, 1984; Průchová et al. 2014) and butterflies (Jiggins  
56 & McMillan, 1997). In fact, they seem to be more common than expected considering that warning  
57 signals are predicted to be under positive frequency-dependent selection (Müller, 1878; Ruxton,  
58 Sherratt, & Speed, 2004). One possible explanation for the co-occurrence of several warning signal  
59 forms within the same population is predator generalization. This refers to a predator's ability to  
60 transfer its learned avoidance of one signal to other signal(s) that share common characteristics  
61 (Gamberale-Stille & Tullberg, 1999; Lindström, Alatalo, Mappes, Riipi, & Vertainen et al. 1999b;  
62 Guilford & Dawkins, 1991; Mappes & Alatalo 1997). Generalization can be symmetric, meaning  
63 that once one colour is learned it is equally possible to transfer the learned aversion to other similar  
64 colours, or asymmetric, implying that transferring a learned avoidance from one colour to other(s)  
65 depends on the signal salience (Aronsson & Gamberale-Stille, 2008; Exnerová et al., 2006;  
66 Gamberale & Tullberg, 1996; Gamberale-Stille & Tullberg, 1999; Ham, Ihalainen, Lindström &  
67 Mappes, 2006; Ruxton, Franks, Balogh, & Leimar, 2008; Waldron et al., 2017).

68         Predator learning involves different cognitive processes that establish the association  
69 between warning coloration and unprofitability, and aid the memorization of this association once  
70 established. This learning process may vary between predators even at intraspecific levels (e.g.  
71 Adamová-Ježová et al., 2016; Endler & Mappes, 2004; Exnerová et al., 2010; 2015; Karlíková et  
72 al., 2016; Lindström, Alatalo, & Mappes, 1999a; Sherratt & Macdougall, 1995; Skelhorn, Halpin,  
73 & Rowe, 2016). Predators may also vary in their ability to cope with defended prey, due for  
74 example to dietary conservatism (Marples & Kelly, 1999; Mettke-Hofmann et al., 2002; Webster &

75 Lefebvre, 2000; Turini, Veselý & Fuchs, 2016). Therefore, investigating how predators learn to  
76 associate the appearance of prey with the noxious effects of their unprofitability is crucial to  
77 understanding how signal variation can be maintained within a population. During the learning  
78 process predators acquire information about the nutrient and toxin content of aposematic prey.  
79 Thus, individual predators are expected to make different decisions on how to use the information  
80 gathered from an encounter with aposematic prey (Exnerová et al., 2003; Exnerová et al., 2007;  
81 Halpin, Skelhorn, & Rowe, 2014; Lynn, 2005; Skelhorn et al., 2016; Trimmer et al., 2011), and  
82 modify their ingestion of toxic prey according to their toxic burden (Skelhorn & Rowe, 2007).

83         Generalized avoidance should be broad and persist for a relatively long time to offer  
84 protection to different warningly coloured prey morphs. On the other hand, naïve predators can also  
85 avoid warningly coloured prey due to innate wariness, neophobia or dietary conservatism  
86 (Exnerová et al., 2007; Lindström, Alatalo & Mappes, 1999; Marples & Kelly, 1999; Marples &  
87 Mappes 2011), which could be further reinforced by the short-term effects of negative experience  
88 with other aposematic prey. It has been suggested that multiple modalities of warning signals can  
89 help predators discriminate between palatable and unpalatable prey (Siddall & Marples, 2008,  
90 Kazemi, Gamberale-Stille & Leimar, 2015). However, generalized avoidance of aposematic prey  
91 can also be based on cues of different sensory modalities, such as odour, sound, colour or pattern or  
92 combinations of these. Depending on the cognitive processes of predators, they could also associate  
93 their negative experience with certain stimuli to any other stimuli encountered simultaneously  
94 (Mackintosh, 1975; Pavlov, 1927). These results emphasize the importance of studying how  
95 multiple cues and separate signal components influence a predator's decision to attack prey  
96 (Kikuchi, Mappes, Sherratt & Valkonen, 2016; Rowe & Halpin, 2013).

97         Here, we tested the hypothesis that the hindwing colour polymorphism of an aposematic  
98 moth is enabled by predator generalization, and investigated whether or not that generalization is  
99 symmetric. We exposed paper models of the different hindwing colour morphs of the wood tiger

100 moth, *Arctia plantaginis* (formerly known as *Parasemia plantaginis*) to natural predators (blue tits,  
101 *Cyanistes caeruleus*), and examined whether, once they learned to avoid one of the colour morphs,  
102 they would generalize this aversion to the two unlearned colour morphs, which would allow  
103 multiple morphs to coexist. A lack of generalization among colour morphs would mean that birds  
104 pay more attention to colours than to other cues of the moth wings.

105

## 106 <H1>METHODS

107 The wood tiger moth (Rönkä, Mappes, Kaila, & Wahlberg, 2016) is an aposematic diurnal moth  
108 with a Holarctic distribution (Hegna, Galarza, & Mappes, 2015). They have two different chemical  
109 defences, one of which is secreted from the prothoracic glands. Although the chemical composition  
110 is not fully known, these fluids contain two types of methoxypyrazines, which are produced de  
111 novo (Burdfield-Steel, Pakkanen, Rojas, Galarza & Mappes 2016) and make them a deterrent to  
112 birds. Experiments with bird predators suggest that the fluids of yellow males have a more repulsive  
113 odour (Rojas et al. 2017), while those of white males taste worse (Rojas, Burdfield-Steel & Mappes  
114 2015). Individuals vary in the degree of melanization and black patterning of the wings, as well as  
115 in levels of chemical defence, but the most striking feature of the wood tiger moth is its local  
116 hindwing colour polymorphism (Hegna et al., 2015). In Europe, its forewings present a black and  
117 white pattern in both males and females, whereas the hindwing colour combined with black pattern  
118 differs between the sexes (e.g. Galarza, Nokelainen, Ashrafi, Hegna, & Mappes, 2014; Hegna &  
119 Mappes, 2014). The distinct white and yellow male morphs are genetically determined by one  
120 autosomal locus and at least three alleles, dominant white, recessive white and intermediate yellow  
121 (Galarza, Nokelainen & Mappes 2016), while female hindwing coloration varies continuously from  
122 yellow to red (Lindstedt et al. 2017; Fig. 1). In Finland, for example, yellow and white males may  
123 occur within one population (Nokelainen, Valkonen, Lindstedt, & Mappes, 2014) whereas female  
124 hindwing coloration is mostly red (Hegna et al., 2015).

125 To study the reaction of bird predators (see below for details on procedure) to the different  
126 hindwing colour morphs, we used artificial moth models. The usage of artificial prey allows for the  
127 controlled manipulation of one or more warning signal components at a time, while accounting for  
128 how predators (i.e. birds) would see them (Endler & Mielke 2005). In this way, other components  
129 can be kept constant and independent of prey qualities, such as the variation in the level of chemical  
130 defence or behaviour (Karlíková et al., 2016; Lindström et al., 1999a; Veselý & Fuchs 2009). Here,  
131 our artificial moth models eliminated individual variation in moth size, shape, degree of  
132 melanization, wing pattern, wing posture, behaviour, smell or taste. Model wings were constructed  
133 with the software GIMP (2.8.16; <http://www.gimp.org/>) from pictures of a real male wood tiger  
134 moth specimen collected in Finland. Pictures of one forewing and one hindwing of a typical white  
135 moth were duplicated to obtain a symmetric pattern for the whole model. The melanization pattern  
136 of the moths used was a representative sample of a wing pattern in Finland (Fig. 1). To control for  
137 the amount and shape of melanized (mainly black) pattern of the wings, yellow and red models  
138 were created from the same wing picture, changing the hue of the white parts of the hindwing  
139 towards yellow or red. Finished models were printed double sided (HP Color LaserJet CP2025) on  
140 waterproof (Rite In The Rain, Tacoma, WA, U.S.A.) paper. To ensure that the model colours  
141 resembled the real wood tiger moth morphs, colour reflectance was measured with an Ocean Optics  
142 Maya2000 Pro spectrometer and average reflectance curves from three spots in the model hindwing  
143 coloration were compared to average reflectance curves of white, yellow and red moth hindwings  
144 (Fig. 1). Models were then cut out from the paper and completed with a body made of rolled pastry,  
145 composed of two parts of lard, six parts of coarse wheat flour and one part of water to make them  
146 edible. The total body weight was  $0.04 \pm 0.005$  g. Bodies were dyed on top with black food  
147 colouring, to make models resemble the real moths as accurately as possible. Finally, bodies were  
148 glued on the paper models with nontoxic glue (UHU stick).

149

## 150 <H2>*Bird predators*

151 Blue tits were chosen as predators for several reasons: (1) they are visual foragers and their visual  
152 capabilities are well known (Hart, Partridge, Cuthill, & Bennett, 2000; Hart & Vorobyev, 2005),  
153 ensuring that they are able to distinguish all of the wood tiger moth's colour morphs; (2) they have  
154 been used in several experiments on coloration (e.g. Dimitrova & Merilaita, 2010; Exnerová et al.,  
155 2007; Kikuchi et al., 2016) and wood tiger moths (Nokelainen, Hegna, Reudler, Lindstedt, &  
156 Mappes, 2012), and also with similar moth models (Rojas, Burdfield-Steel & Mappes 2015); (3) tits  
157 are likely to be important natural predators of wood tiger moths in Finland (Nokelainen, Valkonen,  
158 Lindstedt, & Mappes, 2014); and (4) blue tits are common in central Finland, and easy to capture  
159 and keep in captivity for a short period of time.

160         The birds used for the experiment were caught from Konnevesi Research Station and City of  
161 Jyväskylä (central Finland), maintained individually in plywood cages with a perch, water bowl and  
162 food ad libitum, and kept on a 12:12 h light:dark cycle. Each bird was weighed before and after the  
163 experiment, ringed, and its sex and age were determined before being released to the same place of  
164 capture. Birds were used with permission from the Central Finland Centre for Economic  
165 Development, Transport and Environment and licensed from the National Animal Experiment  
166 Board (ESAVI/9114/04.10.07/2014) and the Central Finland Regional Environment Centre  
167 (VARELY/294/2015). All experimental birds were used according to the ASAB/ABS Guidelines  
168 for the treatment of animals in behavioural research and teaching.

169

## 170 <H2>*Experimental procedures*

171 The experiment consisted of three phases: a preference test, a learning test and a generalization test  
172 (see details below). Each bird was tested individually and only once for each part of the experiment.  
173 The experiment was conducted between November 2015 and March 2016 at Konnevesi Research



174 Station, in central Finland and lasted, on average, 3 days for each individual, depending on how  
175 long the bird took to complete the different tests.

176 Trials took place in experimental custom-built plywood cages (50x50 cm and 70 cm high)  
177 illuminated with a daylight lamp (Exo Terra Repti Glo 10.0 UVB, <http://exo-terra.com/>). Each  
178 aviary had a perch and a water bowl (access ad libitum). Birds were observed through a small  
179 mesh-covered window situated on the front of the cage, and filmed with a Canon Powershot S120  
180 camera. The experiment took place in a dark room to minimize observers disturbing the birds.

181 Food and experimental models were offered on a green platform through a moveable tray  
182 behind a visual barrier, allowing us to estimate the exact time when the bird first saw the model and  
183 thus started the trial (see details in Nokelainen et al. 2012). A standard green background was used,  
184 because wood tiger moths rest on green leaves in nature (Hegna et al. 2013, Nokelainen et al.,  
185 2012). All colours used in the moth models are easily distinguished from the background by birds:  
186 Hegna et al. (2013) reported just noticeable difference (JND) values in colour contrast ranging from  
187 8.6 to 11.6 for white and yellow artificial moth models and real wood tiger moths against the green  
188 background used also in this experiment, and Lindstedt et al. (2011) calculated JND values above  
189 27.27 for orange and red females on natural green leaves of *Alnus incana*.

190 During pretraining, birds were allowed to habituate to the experimental cages and learned to  
191 eat three sunflower seeds from the green platform. To motivate the birds to attack the moth models  
192 during the experiment (see below), they were food deprived for 2 h before the preference test, 1 h  
193 before the learning test and 1 h before the generalization test. After food deprivation, bird  
194 motivation was tested with a sunflower seed; if eaten, the bird was considered ready to begin the  
195 test.

196

197 <H3>Phase 1: Preference test

198 A preference test was included in the experimental protocol for two reasons. As we used wild-  
199 caught birds, we first tested whether they had any pre-existing biases towards white, yellow or red  
200 moth morphs. Second, by offering palatable morphs several times to birds we ensured that any  
201 potential unlearned or learned biases disappeared, allowing us to test the effect of the coloration on  
202 learning and generalization (Ghirlanda & Enquist, 2003).

203 All three morphs (white, yellow and red) were offered simultaneously on the green platform  
204 for 5 min, starting from when the bird first saw them. If the bird did not attack (i.e. grab or peck)  
205 any of the edible model pastry bodies during the 5 min, the models were taken away and presented  
206 again after a break. Once the first attack was made, the models were kept in the cage until the bird  
207 finished eating all the pastry bodies. To ensure that all birds had an equally rewarding experience  
208 with all the colours, we let the birds finish eating the pastry bodies of all models in three  
209 consecutive trials during the preference test. Between the trials, the presentation (order) of the  
210 models on the platform was always changed (Fig. 2).

211 As birds were hesitant to attack the moth models for the first time (hesitation times varying  
212 from 17 s to 2 h consisting of 5 min presentations), we did not use time to attack in analyses.  
213 Instead, we recorded the order in which the models were attacked and eaten during the three  
214 consecutive trials. We compared the order of attacks between the first and the last preference test to  
215 be sure that all the birds got rid of any potential bias in preferences before the learning phase.  
216 Preference test presentations were continued for a maximum of 2 days. Eight of 53 birds did not  
217 attack or finish eating the artificial moth models offered during the preference test and were,  
218 therefore, excluded from further tests.

219

### 220 <H3>Phase 2: Learning test

221 In the second phase of the experiment we tested whether blue tits learn to avoid white, yellow and

222 red models differently, and established learned avoidance towards one of the colour morphs before  
223 the following generalization test. Birds that completed the preference test were divided into three  
224 groups for avoidance learning: 15 birds were offered white models, 15 yellow models and 16 red  
225 models as unpalatable. Groups of birds were selected as similar as possible (i.e. similar sex, age and  
226 size distribution) and birds from all groups were tested simultaneously. All models were made  
227 unpalatable by replacing the water in pastry bodies with 15% chloroquine diphosphate solution  
228 (Sigma Life Science, St Louis, MO, U.S.A.). As the pastry bodies were coloured with black dye on  
229 top, we also added 15% chloroquine diphosphate solution on top of the bodies and let it dry before  
230 the following trials. Chloroquine solution was used because it is odourless (Hong 1976) and thus all  
231 qualities other than palatability (i.e. taste) of the prey items remained the same throughout the  
232 experiment.

233         During the learning test, unpalatable models were presented individually in consecutive  
234 trials alternating with sunflower seeds (Fig. 2). Sunflower seeds were offered to monitor the birds'  
235 motivation to forage and avoid unnecessary starvation. If the bird did not attack the sunflower seed,  
236 it got a 10 min break without food and was then offered a sunflower seed again. If the bird attacked  
237 the sunflower seed, the next unpalatable model was offered 2 min after the bird finished eating. As  
238 long as the bird attacked the models, trials were continued alternating with sunflower seeds. If the  
239 bird did not attack the unpalatable model, but ate the sunflower seed, it was considered to reject the  
240 model. After a bird did not attack the moth model the second time in a row, a small live mealworm  
241 (< 20 mm *Tenebrio molitor* larva) was offered instead of the sunflower seed to test the bird's  
242 motivation to attack insect prey and increase its motivation to forage. If the bird now attacked the  
243 unpalatable model offered after the mealworm, trials were continued again alternating with  
244 sunflower seeds, but if it rejected the unpalatable model, it got another mealworm (Fig. 2). We  
245 considered the bird to have learned to avoid the unpalatable models when it refused to attack three  
246 models in a row, but consumed the sunflower seeds and mealworms offered in between and after

247 the rejected models.

248           Presentation time was set to 5 min from when the bird first saw the model for the first three  
249 trials to make sure that each bird had the opportunity to attack and taste the model. To keep the  
250 overall duration of the generalization experiment within the permitted 4-day limit, a maximum of  
251 30 presentations divided into 2 days was set for the avoidance learning. Furthermore, we reduced  
252 the presentation time to 2 min from the fourth to the sixth trial, and to 1 min for the rest of the trials.  
253 Based on our observations during a pilot experiment with six birds and fixed durations of trials,  
254 birds were unlikely to attack the model and did not consume it within 5 min if they did not attack  
255 within the first minute. Sunflower seeds were usually attacked quickly, and hesitation time declined  
256 to a few seconds as the trials proceeded, implying that 1 min was sufficient to test the bird's  
257 willingness to attack the models. Two of the 46 birds did not stop attacking (white and red) models  
258 within 30 presentations and were therefore excluded from the following generalization test.

259

### 260 <H3>Phase 3: Generalization test

261 In the third phase, we tested whether the 44 birds that had learned one of the colour morphs as  
262 unpalatable would avoid attacking the other two colour morphs. When birds had completed the  
263 learning test, half of them had a break of at least 2 h with food and 1 h of food deprivation before  
264 the last phase of the experiment, and half were tested the following day. The generalization test  
265 started after the bird had consumed a sunflower seed offered to test its motivation to attack. Birds  
266 were tested for the generalization with the colours that they did not learn as unpalatable: yellow and  
267 red for those that learned white as unpalatable, white and red for those that learned yellow, and  
268 white and yellow for those that learned red (Fig. 2). The two colours tested were presented  
269 simultaneously on the green platform in alternating positions for three trials lasting 5 min each. This  
270 allowed us to test the repeatability of bird behaviour. The trials were interspersed with sunflower  
271 seed presentations to make sure that birds were not attacking the models due to lack of motivation.

272 Moreover, offering alternative food ensured that birds were not forced to eat the models simply  
273 because of hunger. Models used for the generalization test were palatable.

274

275 <H2>*Statistical analysis*

276 <H3>*Phase 1: Preference test*

277 The potential colour bias of blue tits was analysed separately for the first trial (Fig. 2) and all three  
278 trials pooled. Colour biases are most likely to be detected reliably by checking the order of attacks  
279 on the white, yellow and red models in the first trial ( $N=53$ ), when the birds first encountered the  
280 models. The number of moths of each colour taken first, as well as left last, were compared by  
281 means of a chi-square test. Additionally, all three trials were pooled in another analysis to find out  
282 whether the potential biases disappeared as the birds learned to eat all the models. In the pooled  
283 data, each colour was scored based on the order of choice by the bird in each trial; the colour  
284 chosen first was scored 1, that chosen second was scored 2 and the colour chosen last was scored 3.  
285 Thereby, the minimum score expected for a preferred colour was 3 (i.e. always chosen first), and  
286 the maximum score expected for an avoided colour was 9 (i.e. always chosen last). To study the  
287 population level bias to all colours, the scores of each colour in each of the three trials were  
288 summed and compared to an even distribution by means of a chi-square test. The potential  
289 influence of the first colour chosen on the subsequent choice was checked with a binomial exact  
290 test.

291

292 <H3>*Phase 2: Learning test*

293 Potential differences in learning rate between the three colour morphs were analysed using a mixed-  
294 effect Cox regression model, using the 'coxme' package (version 2.2-5; Therneau, 2015) in RStudio  
295 (v. 0.99.902; RStudio, 2015). The response variable was the probability that the presented model

296 was attacked in each trial; time was represented as number of trials. Model colour was added as an  
297 explanatory factor and bird individual as a random effect.

298

### 299 <H3>Phase 3: Generalization test

300 If birds generalized their learned aversion of a given colour to the two nonlearned colours, we  
301 would expect them to refrain from attacking models offered during the generalization test but eat  
302 the alternative prey offered between trials. Hence, the probability of attack on palatable models is  
303 expected to be significantly lower than random ( $< 0.5$ ). If, in contrast, birds were unable to  
304 generalize their learned avoidance, we would expect the attack probability to be significantly higher  
305 than 0.5. High attack probability is expected (in the case of no generalization) since the birds had  
306 attacked and eaten similar palatable models in the preference test and did attack the models  
307 presented first in the learning test within the 5 min presentation. Thus, to test whether the birds  
308 generalized and the attack probability on the models was lower (or higher) than random, we built  
309 two generalized linear mixed models (GLMM 1 and 2) with a logit link and binomial distribution,  
310 including whether the prey was attacked (1) or not (0) as the dependent variable. Bird ID and bird  
311 ID nested within trial in GLMM 2 were added as random factors using package lme4 (Bates et al.  
312 2015) in R. GLMM 1 was used to test for generalization in the first trial only, and GLMM 2 in all  
313 three trials.

314 To test for asymmetric generalization, we divided the birds into six treatment classes by the  
315 colours they learned (white, yellow and red) and were offered (yellow and red, white and red, white  
316 and yellow, respectively). This classification was then used as the explanatory variable ('colour  
317 combination') in two GLMM models separately for the first trial only (Table A1 in the Appendix)  
318 and all three trials (Table A2 in the Appendix) of generalization (again with a logit link and  
319 binomial distribution, including whether the prey was attacked (1) or not (0) as the dependent  
320 variable, and bird ID nested within trial and/or bird ID as random factors). A chi-square test was

321 used to check whether the birds attacked one colour morph first more frequently between the two  
322 colour morphs offered, both in the first trial and in the first three trials pooled (Table A3 in the  
323 Appendix). Birds tested the same or the following day after avoidance learning were pooled in all  
324 analyses, as there were no differences in the number of attacks between birds tested the same or the  
325 following day after avoidance learning in the first trial (unpaired two-sample Wilcoxon test:  
326  $W=276$ ,  $N=44$ ,  $P=0.21$ ) or in the three trials pooled ( $W=262$ ,  $N=44$ ,  $P=0.59$ ). We also checked  
327 whether the rate of learning correlated with the number of attacks in the generalization test with a  
328 Spearman correlation.

329

## 330 <H1>RESULTS

### 331 <H2>Preference test

332 At the population level, birds did not show any preferences (Table 1) or aversion (chi-square test:  
333  $\chi^2_2=2.577$ ,  $P=0.28$ ) towards any of the colours (white, yellow or red) during the first trial. Birds  
334 chose the second colour to attack with the same probability between the two colours left,  
335 irrespective of the first colour chosen (binomial exact test:  $P>0.05$  for all comparisons).

336 At the individual level, 35 birds (85.4%,  $N=41$ ) chose at least one colour in the same order  
337 for two different trials (for instance, the same bird chose the yellow morph as last choice in two  
338 trials out of three). Two birds showed a strong preference for one of the colour morphs, choosing  
339 the same colour (yellow and red, respectively) first for all three trials. Three birds showed  
340 avoidance for one colour morph (one for white, two for red), leaving the same colour as last in all  
341 the trials. All other birds changed their order of choice during the three trials, showing that they got  
342 rid of potential biases towards the colours during training. When we tested the overall scores for  
343 each colour morph during the three trials, birds did not show differences between the colour morphs  
344 (chi-square test:  $\chi^2_2=0.789$ ,  $P=0.67$ ).

345

346 <H2>*Learning test*

347 Apart from two individuals, all birds ( $N=44$ ) learned to avoid their moth model according to the  
348 criterion of no attack over three subsequent trials. The number of trials needed to learn to avoid the  
349 unpalatable model varied between 2 and 23 among the birds (mean=7). The Cox regression model  
350 (Fig. 3) showed that birds learned to avoid the red colour morph significantly faster than the yellow  
351 ( $Z=2.17$ ,  $P=0.03$ ), but showed no significant differences between the yellow and white morphs  
352 ( $Z=0.87$ ,  $P=0.38$ ).

353

354 <H2>*Generalization test*

355 Overall, blue tits did not generalize their learned avoidance from one colour morph to the other two,  
356 as the attack probabilities were significantly higher than 0.5 in the first trial (GLMM 1:  $Z=4.33$ ,  
357  $P<0.001$ ; Fig. 4) and the three trials pooled (GLMM 2:  $Z=6.42$ ,  $P<0.001$ ). Only three of 44  
358 individuals did not attack any of the palatable models during the generalization test, showing  
359 generalized avoidance.

360 We did not find clear evidence of asymmetric generalization. The estimated attack  
361 probabilities did not differ significantly between the combinations of colour learned and colour  
362 offered in generalization trials (Tables A1 and A2 in the Appendix), and no differences were found  
363 in which colour the birds attacked first during the first trial of the generalization test (chi-square  
364 test:  $P>0.05$  in all cases; Table A3 in the Appendix). In the first trial, however, birds that learned  
365 yellow attacked fewer white models compared to the other colour combinations, and the effect is  
366 near the 0.05 significance level (Table A1 in the Appendix). Also, when the three trials were  
367 pooled, we found that birds that learned to avoid the white morph attacked the red morph first  
368 significantly more often than the yellow one (chi-square test:  $\chi^2_1=5.9$ ,  $P=0.02$ ).



369           The rate of learning did not correlate significantly with the proportion of attacked models in  
370 the first generalization trial (Spearman correlation  $r_s=0.12$ ,  $N=44$ ,  $P=0.45$ ) or the total number of  
371 attacks in the three generalization trials (Spearman correlation:  $r_s=0.27$ ,  $N=44$ ,  $P=0.07$ ), thus  
372 allowing us to compare the effect of the colour learned on generalization despite different learning  
373 rates of red versus the other colours.

374

## 375 <H1>DISCUSSION

### 376 <H2>*No generalization based on hindwing colour*

377 Generalized avoidance by local predators from one warning signal to another has been proposed to  
378 contribute to the maintenance of local warning signal polymorphism in aposematic species  
379 (Amézquita et al., 2013; Exnerová et al., 2006; Gamberale & Tullberg, 1996; Gamberale-Stille &  
380 Tullberg, 1999; Ham et al., 2006; Hegna & Mappes, 2014; Rojas, Rautiala, & Mappes, 2014;  
381 Ruxton et al., 2008; Waldron et al., 2017). Here we studied in more detail how bird predators learn  
382 and generalize the warning colours of a polymorphic (red, yellow, white) wood tiger moth  
383 population using artificial moth models. Attack rates during the generalization test were in general  
384 very high. Indeed, the birds did not generalize their learned avoidance among the wood tiger moth  
385 morphs, but instead treated them as different prey types based on the differing hindwing colour  
386 alone, as the morph models used did not differ in size, shape, pattern, taste or smell.

387

### 388 <H2>*The importance of colour*

389 Our findings are in line with previous experiments showing that colour is of foremost importance in  
390 avian predator learning, contributing especially to the discrimination between palatable and  
391 unpalatable prey (Aronsson & Gamberale-Stille, 2008, Kazemi, Gamberale-Stille, Tullberg, &  
392 Leimar, 2014). A large body of research has demonstrated birds' ability to learn to avoid

393 conspicuous, unpalatable prey (Aronsson & Gamberale-Stille, 2008; Rowe, Lindström, & Lyytinen,  
394 2004; Svádová et al., 2009). This is because conspicuous warning coloration enhances prey  
395 recognition (Guilford, 1986; Sherratt & Beatty, 2003), speed of avoidance learning and  
396 memorability (e.g. Roper & Redston, 1987). Different predators may use different components of  
397 the warning signal as a primary cue depending on their sensory systems (Aronsson & Gamberale-  
398 Stille, 2012; Endler, 1992; Guilford & Dawkins, 1991) and disregard others. Studies done with  
399 birds have demonstrated that colour seems to be a more important feature in warning signals than  
400 size or pattern (Aronsson & Gamberale-Stille, 2008; Exnerová et al., 2006; Sillén-Tullberg, 1985;  
401 Terhune, 1977).

402         As predators can associate palatability or unpalatability with several different kinds of prey  
403 traits, it is convenient to compare the relative importance of those traits with how much they  
404 facilitate associative learning. The expectation is that more salient signals are learned faster  
405 (Kazemi et al. 2014). Our results indicate that red was the most salient warning colour: birds  
406 learned to avoid the red morph faster than the other morphs. This is in accordance with Lindstedt et  
407 al. (2011), who found that the red female morph of the wood tiger moth was better protected against  
408 bird predators, suffering fewer attacks than its orange or yellow counterparts. Indeed, red has been  
409 shown to be a very efficient warning signal compared to other warning colours such as orange,  
410 yellow or white, and other colours such as violet, blue, green and brown, at least for some bird  
411 predators (Cibulková, Veselý, & Fuchs, 2014; Exnerová et al., 2006; Gamberale-Stille & Tullberg,  
412 1999; Lindstedt et al., 2011; Svádová et al., 2009).

413         In the present study prey items were made to resemble real wood tiger moth morphs as  
414 closely as possible, keeping all traits other than hindwing colour constant. This allowed us to  
415 compare the effects of warning coloration of hindwings only. Changing the warning colour hue  
416 altered not only the internal contrast on model hindwings, but also the contrast between the model  
417 and the green background. Although all colours in our experiment were clearly conspicuous to the

418 birds, red had the highest colour contrast against the green background whereas white had the  
419 lowest. This might explain why red seems to be the most salient signal. Aronsson and Gamberale-  
420 Stille (2009) found similar results using domestic chicks, *Gallus gallus domesticus*, which learned  
421 to avoid red prey faster if presented on a contrasting background compared to a background of  
422 similar hue. In another experiment, however, red prey colour was found to influence predator  
423 avoidance independent of background colour (Sillén-Tullberg, 1985). Thus, it seems that both prey  
424 coloration per se and its contrast against the background can contribute to predator avoidance, but it  
425 is still relatively unclear which properties of prey coloration, chromatic or achromatic, play the  
426 most important role. Previous work with wood tiger moths has shown that the achromatic contrast  
427 against a green background is highest for white morphs, which are the most luminous of the three  
428 (Lindstedt et al. 2011; Henze, Lind, Mappes, Rojas & Kelber 2017). Luminance has not been found  
429 to affect predator responses towards the wood tiger moth, while the chromatic contrast in hue seems  
430 to be very important (Nokelainen et al., 2012).

431         Generalization has been suggested to stabilize selection towards aposematic signals via a  
432 peak shift phenomenon (Leimar, Enquist, & Sillen-Tullberg, 1986; Lindström et al. 1999b). The  
433 minimum (and maximum) responses of predators (i.e. peaks of the generalization gradient) have  
434 been found to be displaced from the negative (and positive) stimulus (Gamberale & Tullberg, 1996;  
435 Hanson, 1959), such as yellow, towards a similar, but more salient novel stimulus, such as red.  
436 Overall, we did not find strong evidence of asymmetric generalization, but there were some trends  
437 between the colours tested. Birds that learned to avoid red models attacked almost all the white and  
438 yellow models in the generalization trials, whereas birds that learned the less salient colours yellow  
439 and white generalized more, hinting at a tendency to generalize from the less salient signals towards  
440 the more salient signal.

441         Svádová et al. (2009) found asymmetric generalization using great tits, *Parus major*, which  
442 did not generalize from red firebugs, *Pyrrhocoris apterus*, to white or yellow mutants, but did

443 generalize from yellow mutants to red firebugs. Interestingly, in our experiment, four blue tits that  
444 learned to avoid the yellow morph ( $N=15$ ) refrained from attacking white models, while only two  
445 did so for the red ones. Birds that learned to avoid the white morph attacked both unlearned morphs  
446 equally, but chose red models first more often than yellow ones. This indicates that birds tended to  
447 generalize more between the white and yellow than between white and red. The yellow morph  
448 seems to benefit least from the other colours, since only between 7 and 34% of yellow models were  
449 left unattacked (Table 2, Fig. 4).

450

## 451 <H2>Limitations of testing generalization in the laboratory

452 Despite the majority of birds showing no generalization in our experiment, the possibility remains  
453 that predators might generalize among morphs of the wood tiger moth under different  
454 circumstances. Studying generalization in the wild is practically impossible due to the rareness of  
455 predation events on aposematic prey as well as difficulties in observing the choices of individual  
456 predators in natural conditions. A previous study aiming to explain the variability in the warning  
457 signals of the harlequin poison frog, *Dendrobates histrionicus*, showed that predators avoided  
458 attacking aposematic frog models but not cryptic ones in areas where aposematic frogs occur,  
459 exhibiting some generalization among different frog colour morphs in the field. However, the same  
460 study found no generalization by naïve chicks tested in the laboratory (Amézquita et al., 2013). This  
461 might imply that naïve and experienced predators in the wild can use different generalization  
462 strategies (see also Ihalainen, Lindström, Mappes & Puolakkainen, 2008). Birds might also be  
463 prone to generalize more or less widely under different circumstances (Aronsson & Gamberale-  
464 Stille, 2012), for example under physiological stress during winter months (Barnett, Bateson &  
465 Rowe 2007; Chatelain et al. 2013; Veselý et al. 2017), limited food availability (Lindström, Alatalo,  
466 Lyytinen & Mappes, 2004; Ihalainen, Rowland, Speed, Ruxton, & Mappes, 2012), limited time to  
467 make decisions (Ings & Chittka, 2008), when the prey is dangerously toxic (Lindström, Alatalo, &

468 Mappes, 1997; Sherratt, 2002), when the prey community is complex versus simple (Ihalainen et  
469 al., 2007), or when the prey population has palatable Batesian mimics in addition to the unprofitable  
470 prey (Plowright & Owen, 1980).

471         Avoidance learning has been suggested to happen in two steps: first, the birds learn simple  
472 rules based on certain cues, and once the basic rules are formed, they then learn in more detail about  
473 prey quality (Chittka & Osorio, 2007). Recent studies indicate that birds are able to assess the  
474 nutritional benefits of unprofitable prey and use this information in subsequent encounters (Halpin  
475 et al., 2014). This ability could have affected not only bird learning rates, but also their decision to  
476 attack in the generalization phase of our experiment. As our models' pastry bodies were of high  
477 nutritional value and the birds were hungry, it is possible that the birds were willing to take more  
478 risks and thus took more trials to learn to avoid the models than it would take them to learn to avoid  
479 defended prey in the wild. In addition, the 5 min presentations gave the birds plenty of time to  
480 decide whether to attack or not, and to make more sophisticated assessments of prey quality than  
481 might be possible in the wild. Birds were given alternative food between the presentations, but not  
482 enough for saturation, and would thus have benefited energetically from discriminating between the  
483 unprofitable and profitable models. Nevertheless, the cost–benefit relationship was exactly the same  
484 for all morphs in our experiment and, thus, we can safely compare the relative differences between  
485 morphs in their salience.

486         The avoidance learning was based on counterconditioning, where the colour signal was first  
487 associated with a positive reinforcement (i.e. palatability) and then with a negative reinforcement  
488 (i.e. unpalatability). Previous research has shown that in cases of single counterconditioning the  
489 associations learned second are forgotten at higher rates than those learned first (Speed, 2000 and  
490 references therein). Therefore, it is possible that the birds' experience and learned association with  
491 palatability in the preference test exceeded the effect of generalized avoidance among the morphs  
492 for most of the birds, which could partly explain the low level of generalization observed. Offering

493 the models as palatable at the beginning of the experiment was necessary to get rid of any pre-  
494 existing biases or neophobia prior to learning and testing generalization effects of the birds; this was  
495 also necessary to motivate the birds to attack and taste the unpalatable models during the first  
496 learning trials.

497 In the preference and generalization tests, simultaneous prey choice was used to decrease  
498 the numbers of birds needed to accomplish the experiment. Simultaneous prey choice is also a very  
499 powerful set-up to detect any potential predator biases but, obviously, this approach has  
500 disadvantages too (Fig. 2). For example, it is possible that long hesitation delays during the first  
501 presentation of the preference test were partly due to an aggregation effect, as aggregations of  
502 conspicuous prey have been found to be aversive to predators (Gamberale-Stille, 2000; Riipi,  
503 Alatalo, Lindstrom, & Mappes, 2001). On the other hand, Nokelainen et al. (2012) presented wood  
504 tiger moths singly to birds, several of which also hesitated for a long time before attacking them.  
505 Thus, it is difficult to say how much the simultaneous presentation influenced our results, but  
506 during the flying season wood tiger moth morphs typically aggregate at the same sites. Males of  
507 both morphs are often found near calling females, and thus all morphs can be visible and vulnerable  
508 to predators simultaneously.

509 Lastly, if the wood tiger moths are able to survive bird attacks, the use of artificial models  
510 does not necessarily give an accurate estimate of selection. A considerable proportion of attacks in  
511 the generalization test were just a single peck, leaving the models uneaten, and thus not necessarily  
512 'killed'. The birds' willingness to attack but reluctance to consume the models could stem from the  
513 psychology of birds' decision making (Marples & Kelly, 1999). Adamová-Ježová et al. (2016)  
514 showed that for great tits and coal tits, *Parus ater*, neophobia (i.e. the avoidance of novel prey  
515 affecting the decision to attack), but not dietary conservatism (i.e. restriction of diet to certain prey  
516 types affecting the decision to consume the prey), was deactivated during pretraining with a  
517 palatable prey, but the initial hesitation of blue tits was not affected by earlier experience. Blue tits

518 have been found to show higher general aversion even towards palatable prey than, for example,  
519 great tits, probably because of higher dietary conservatism (Veselý et al. 2006, 2013, Prokopová et  
520 al. 2010, Turini et al. 2016). This indicates that predators' decisions of whether to attack or not after  
521 avoidance learning might be species specific and, thus, not generalizable from blue tits to other  
522 predators. In our experiment, 45 of 53 blue tits overcame their initial avoidance during the  
523 preference test and attacked and consumed the models readily in the following avoidance trial. As  
524 those birds that did not overcome their hesitation during the preference test were not included in the  
525 following phases of the experiment, our results describe the generalization tendency of the less  
526 hesitant individuals, which are more likely to attack aposematic prey in the wild in the first place.  
527 Many of these less hesitant individuals, however, seemed to regain their dietary conservatism after  
528 they had learned avoidance, as they no longer consumed the palatable models attacked. In  
529 conclusion, whereas no generalization was found regarding the attack probabilities, we did find  
530 individual variation in avoidance learning and dietary conservatism, which could affect selection in  
531 the wild.

532

### 533 <H2>*The importance of other cues*

534 Somewhat surprisingly, the blue tits had no initial biases towards any of the hindwing colours.  
535 Earlier studies on the wood tiger moth have found differential predation pressure in the field  
536 (Lindstedt et al., 2011; Nokelainen et al., 2012; Nokelainen et al., 2014) and different hesitation  
537 times by local predators (Lindstedt et al., 2011; Nokelainen et al., 2012) towards the different  
538 colour morphs. As the differences in hesitation times were found using living moths (Nokelainen et  
539 al., 2012), it is possible that other cues, such as odour, influenced the results. In nature, the wood  
540 tiger moth relies on multiple signal components (i.e. odour, taste) in addition to the visual cues  
541 when exposed to potential predators (Rojas, Burdfield-Steel & Mappes 2015). Its chemical defence  
542 contains pyrazines (Rojas et al. 2017; Burdfield-Steel, Pakkanen, Rojas, Galarza & Mappes 2016),

543 a group of compounds with a characteristic aversive smell, which is effective against birds  
544 (Guilford, Nicol, Rothschild, & Moore, 1987; Rowe & Guilford, 1996). In fact, pyrazine is known  
545 to trigger hidden aversions to red and yellow colours (Rowe & Guilford, 1996) and conspicuous  
546 prey (Lindström, Rowe, & Guilford, 2001), and enhance both learning and memorability of yellow  
547 (Siddall & Marples, 2008) or red coloured prey (Barnea, Gvaryaahu, & Rothschild, 2004) at least in  
548 domestic chicks (Siddall & Marples, 2008). Pyrazine odour has been associated with Müllerian  
549 mimicry rings of insects and suggested to function as a warning signal (Rothschild, 1961). On the  
550 other hand, pyrazine odour has also been shown to assist in discriminating prey and thus reduce  
551 avoidance generalization between differently coloured prey if the odour is present on only some of  
552 them (Siddall & Marples, 2008; Rowe & Guilford, 1996). The specific roles of different cues in  
553 predator–prey interactions are uncertain. It might be that odour is easy to associate with palatability,  
554 but only functions close up, whereas conspicuous colours aid in memorizing which prey to avoid  
555 even from a distance. Here we were interested in colour only. However, it has been shown that  
556 when colour is kept constant, predators can discriminate prey based on pattern (e.g. Prokopová et al.  
557 2010; Veselý et al. 2013), and when both colour and pattern are equal, other visual and/or chemical  
558 features of the prey are used for prey recognition (Karlíková et al. 2016).

559

## 560 <H2>Conclusions

561 Overall, the colour polymorphism of the wood tiger moth in Finland seems unlikely to be  
562 maintained by generalized avoidance based on its warning coloration only. However, predators  
563 were hesitant to attack any of the aposematic morphs in the first place, and if they were to encounter  
564 them in the wild sharing other warning cues such as pyrazine odour, general aversion seems likely  
565 to occur. More knowledge on how predators acquire and use information on prey qualities in  
566 different contexts is needed to conclude whether predator generalization contributes to the  
567 maintenance of multiple aposematic morphs (see also Skelhorn et al. 2016). The possibility that



568 wild predators can generalize on the basis of the pyrazine odour or the combination of colour and  
569 odour requires further investigation. Alternative explanations for the occurrence of local warning  
570 signal polymorphism include negative frequency-dependent natural selection, sexual selection,  
571 frequency-dependent flight activity (Rojas, Gordon, & Mappes, 2015), signal efficacy trade-offs  
572 with other life history traits (Hegna, Nokelainen, Hegna, & Mappes, 2013; Nokelainen et al., 2012),  
573 predator species-specific mortality differences between morphs (Nokelainen et al., 2014) or  
574 combinations of these mechanisms (Gordon, Kokko, Rojas, Nokelainen, & Mappes, 2015). Colour  
575 polymorphism could also be explained by multiple-model mimicry (Edmunds, 2000), if the  
576 different morphs share warning colours with other defended prey species, and predators generalize  
577 their avoidance from one species to the other based on similar coloration. Thus, generalization of  
578 learned avoidance remains as a possible contributor to the maintenance of local polymorphism in  
579 wood tiger moth populations. In conclusion, we argue based on our results that although predator  
580 generalization could well contribute to the maintenance of different aposematic morphs under  
581 certain circumstances, it is unlikely to occur among distinct colour morphs of otherwise similar  
582 prey of visually oriented avian predators.

583

#### 584 **Acknowledgments**

585 We are indebted to Helinä Nisu and Tuuli Salmi for help with the birds at Konnevesi Research  
586 Station, and to Andrés López-Sepulcre, Sebastiano De Bona and Janne Valkonen for their  
587 thoughtful advice on statistical analyses. We also thank the ‘Darwin group’ at the University of  
588 Jyväskylä for discussions and two anonymous referees and the editor for helpful comments on the  
589 manuscript. This study was funded by the Centre of Excellence in Biological Interactions  
590 (Academy of Finland, project no. 284666). C.D.P. was funded by the Erasmus Exchange  
591 Programme.

592

593 **References**

- 594 Adamová-Ježová, D., Hospodková, E., Fuchsová, L., Štys, P., & Exnerová, A. (2016). Through  
595 experience to boldness? Deactivation of neophobia towards novel and aposematic prey in  
596 three European species of tits (Paridae). *Behavioural Processes*, *131*, 24-31. doi:  
597 10.1016/j.beproc.2016.07.014
- 598 Amézquita, A., Castro, L., Arias, M., González, M., & Esquivel, C. (2013). Field but not lab  
599 paradigms support generalisation by predators of aposematic polymorphic prey: the  
600 *Oophaga histrionica* complex. *Evolutionary Ecology*, *27*(4), 769-782. doi: 10.1007/s10682-  
601 013-9635-1
- 602 Aronsson, M., & Gamberale-Stille, G. (2008). Domestic chicks primarily attend to colour, not  
603 pattern, when learning an aposematic coloration. *Animal Behaviour*, *75*, 417-423. doi:  
604 10.1016/j.anbehav.2007.05.006|ISSN 0003-3472
- 605 Aronsson, M., & Gamberale-Stille, G. (2009). Importance of internal pattern contrast and contrast  
606 against the background in aposematic signals. *Behavioral Ecology*, *20*(6), 1356-1362. doi:  
607 10.1093/beheco/arp141
- 608 Aronsson, M., & Gamberale-Stille, G. (2012). Colour and pattern similarity in mimicry: evidence  
609 for a hierarchical discriminative learning of different components. *Animal Behaviour*, *84*(4),  
610 881-887. doi: 10.1016/j.anbehav.2012.07.011
- 611 Barnea, A., Gvoryahu, G., & Rothschild, M. (2004). The effect of the odour of pyrazine and colours  
612 on recall of past events and learning in domestic chicks (*Gallus gallus domesticus*). In H. F.  
613 Van Emden & M. Rothschild (Eds.), *Insect and Bird Interactions* (pp. 205-216). Andover,  
614 U.K.: Intercept Ltd.
- 615 Barnett, C. A., Bateson, M., & Rowe, C. (2007). State-dependent decision making: educated  
616 predators strategically trade off the costs and benefits of consuming aposematic prey.  
617 *Behavioral Ecology*, *18*(4), 645-651. doi: 10.1093/beheco/arm027

618 Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models  
619 Using lme4. *Journal of Statistical Software*, 67, 1-48. doi: doi:10.18637/jss.v067.i01

620 Burdfield-Steel, E., Pakkanen, H., Rojas, B., Galarza, J. A. & Mappes, J. 2016. *De novo synthesis*  
621 *of chemical defences in an aposematic moth*. Submitted manuscript.

622 Chatelain, M., Halpin, C. G., & Rowe, C. (2013). Ambient temperature influences birds' decisions  
623 to eat toxic prey. *Animal Behaviour*, 86(4), 733-740. doi: 10.1016/j.anbehav.2013.07.007

624 Chittka, L., & Osorio, D. (2007). Cognitive dimensions of predator responses to imperfect mimicry.  
625 *PLoS Biology*, 5(12), e339. doi: 10.1371/journal.pbio.0050339

626 Chouteau, M., Arias, M., & Joron, M. (2016). Warning signals are under positive frequency-  
627 dependent selection in nature. *Proceedings of the National Academy of Sciences*, 113(8),  
628 2164-2169.

629 Cibulková, A., Veselý, P., & Fuchs, R. (2014). Importance of conspicuous colours in warning  
630 signals: the great tit's (*Parus major*) point of view. *Evolutionary Ecology*, 28(3), 427-439.  
631 doi: 10.1007/s10682-014-9690-2

632 Dimitrova, M., & Merilaita, S. (2010). Prey concealment: visual background complexity and prey  
633 contrast distribution. *Behavioral Ecology*, 21(1), 176-181. doi: 10.1093/beheco/arp174

634 Edmunds, M. (2000). Why are there good and poor mimics? *Biological Journal of the Linnean*  
635 *Society*, 70(3), 459-466. doi: 10.1111/j.1095-8312.2000.tb01234.x

636 Endler, J. A. (1991). Interactions between predators and prey. In J. R. Krebs and N. B. Davies  
637 (Eds.), *Behavioural ecology* (pp.169-203), 3d ed. London: Blackwell Scientific.

638 Endler, J. A. (1992). Signals, signal condition and the direction of evolution. *American Naturalist*,  
639 139, S125-S153. doi: 10.1086/285308

640 Endler, J. A., & Mappes, J. (2004). Predator mixes and the conspicuousness of aposematic signals.  
641 *American Naturalist*, 163(4), 532-547. doi: 10.1086/382662

642 Endler, J. A., & Mielke Jr, P. W. (2005). Comparing entire colour patterns as birds see them.

643 *Biological Journal of the Linnean Society*, 86(4), 405-431. doi: 10.1111/j.1095-  
644 8312.2005.00540.x

645 Exnerová, A., Landová, E., Štys, P., Fuchs, R., Prokopová, M., & Cehláriková, P. (2003). Reactions  
646 of passerine birds to aposematic and non-aposematic firebugs (*Pyrrhocoris apterus* ;  
647 Heteroptera). *Biological Journal of the Linnean Society*, 78(4), 517-525. doi:  
648 10.1046/j.0024-4066.2002.00161.x

649 Exnerová , A., Svádová , K., Štys, P., Barcalová, S., Landová, E., Prokopová, M., . . . Socha, R.  
650 (2006). Importance of colour in the reaction of passerine predators to aposematic prey:  
651 experiments with mutants of *Pyrrhocoris apterus* (Heteroptera). *Biological Journal of the*  
652 *Linnean Society*, 88, 143-153.

653 Exnerová, A., Štys, P., Fučíková, E., Veselá, S., Svádová , K., Prokopová, M., . . . Landová, E.  
654 (2007). Avoidance of aposematic prey in European tits (Paridae): learned or innate?  
655 *Behavioral Ecology*, 18, 148-156.

656 Exnerová, A., Svádová, K. H., Fučíková, E., Drent, P., & Štys, P. (2010). Personality matters:  
657 individual variation in reactions of naive bird predators to aposematic prey. *Proceedings of*  
658 *the Royal Society of London B: Biological Sciences*, 277(1682), 723-728. doi:  
659 10.1098/rspb.2009.1673

660 Exnerová, A., Jezová, D., Štys, P., Doktorovová, L., Rojas, B. & Mappes, J. (2015). Different  
661 reactions to aposematic prey in 2 geographically distant populations of great tits. *Behavioral*  
662 *Ecology*, 26(5), 1361-1370. doi: 10.1093/beheco/arv086.

663 Galarza, J. A., Nokelainen, O., Ashrafi, R., Hegna, R. H., & Mappes, J. (2014). Temporal  
664 relationship between genetic and warning signal variation in the aposematic wood tiger  
665 moth (*Parasemia plantaginis*). *Molecular Ecology*, 23(20), 4939-4957. doi:  
666 10.1111/mec.12913

667 Galarza, J. A., Nokelainen, O. & Mappes, J. (2016). Genetic architecture of colour polymorphism in

668 the aposematic moth *Arctia plantaginis*. Manuscript in preparation.

669 Gamberale, G., & Tullberg, B. S. (1996). Evidence for a peak-shift in predator generalization  
670 among aposematic prey. *Proceedings of the Royal Society of London Series B: Biological*  
671 *Sciences*, 263(1375), 1329-1334.

672 Gamberale-Stille, G. (2000). Decision time and prey gregariousness influence attack probability in  
673 naive and experienced predators. *Animal Behaviour*, 60, 95-99. doi:  
674 10.1006/anbe.2000.1435

675 Gamberale-Stille, G., & Tullberg, B. S. (1999). Experienced chicks show biased avoidance of  
676 stronger signals: an experiment with natural colour variation in live aposematic prey.  
677 *Evolutionary Ecology*, 13(6), 579-589. doi: 10.1023/A:1006741626575

678 Ghirlanda, S., & Enquist, M. (2003). A century of generalization *Animal Behaviour*, 66, 15–36.

679 Gordon, S. P., Kokko, H., Rojas, B., Nokelainen, O., & Mappes, J. (2015). Colour polymorphism  
680 torn apart by opposing positive frequency-dependent selection, yet maintained in space.  
681 *Journal of Animal Ecology* 84(6), 1555–1564. doi: DOI: 10.1111/1365-2656.12416

682 Guilford, T. (1986). How do ‘warning colours’ work? Conspicuousness may reduce recognition  
683 errors in experienced predators. *Animal Behaviour*, 34, 286-288. doi: 10.1016/0003-  
684 3472(86)90034-5

685 Guilford, T., & Dawkins, M. S. (1991). Receiver psychology and the evolution of animal signals.  
686 *Animal Behaviour*, 42, 1-14.

687 Guilford, T., Nicol, C., Rothschild, M., & Moore, B. P. (1987). The biological roles of pyrazines:  
688 evidence for a warning odour function. *Biological Journal of the Linnean Society*, 31(2),  
689 113-128. doi: 10.1111/j.1095-8312.1987.tb01984.x

690 Halpin, C. G., Skelhorn, J., & Rowe, C. (2014). Increased predation of nutrient-enriched aposematic  
691 prey. *Proceedings of the Royal Society B: iological Sciences*, 281(1781). doi:  
692 10.1098/rspb.2013.3255

693 Ham, A. D., Ihalainen, E., Lindstrom, L., & Mappes, J. (2006). Does colour matter? The  
694 importance of colour in avoidance learning, memorability and generalisation. *Behavioral*  
695 *Ecology and Sociobiology*, 60(4), 482-491. doi: 10.1007/s00265-006-0190-4

696 Hanson, H. M. (1959). Effects of discrimination training on stimulus generalization. *Journal of*  
697 *Experimental Psychology*, 58(5), 321.

698 Hart, N. S., Partridge, J. C., Cuthill, I. C., & Bennett, A. T. D. (2000). Visual pigments, oil droplets,  
699 ocular media and cone photoreceptor distribution in two species of passerine bird: the blue  
700 tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). *Journal of Comparative*  
701 *Physiology A*, 186(4), 375-387. doi: 10.1007/s003590050437

702 Hart, N. S., & Vorobyev, M. (2005). Modelling oil droplet absorption spectra and spectral  
703 sensitivities of bird cone photoreceptors. *Journal of Comparative Physiology A*, 191(4),  
704 381-392. doi: 10.1007/s00359-004-0595-3

705 Hegna, R. H., Galarza, J. A., & Mappes, J. (2015). Global phylogeography and geographical  
706 variation in warning coloration of the wood tiger moth (*Parasemia plantaginis*). *Journal of*  
707 *Biogeography*, 42, 1469–1481. doi: 10.1111/jbi.12513

708 Hegna, R. H., & Mappes, J. (2014). Influences of geographic differentiation in the forewing  
709 warning signal of the wood tiger moth in Alaska. *Evolutionary Ecology*, 28(6), 1003-1017.  
710 doi: 10.1007/s10682-014-9734-7

711 Hegna, R. H., Nokelainen, O., Hegna, J. R., & Mappes, J. (2013). To quiver or to shiver: increased  
712 melanization benefits thermoregulation, but reduces warning signal efficacy in the wood  
713 tiger moth. *Proceedings of the Royal Society B: Biological Sciences*, 280(1755), 20122812.  
714 doi: 10.1098/rspb.2012.2812

715 Henze, M., Lind, O., Mappes, J., Rojas, B. & Kelber, A. (2017). An aposematic colour-  
716 polymorphic moth seen through the eyes of conspecifics and predators – sensitivity and  
717 colour discrimination in *Arctia plantaginis*. Submitted manuscript.

- 718 Hong, D. D. (1976). Chloroquine phosphate. *Analytical profiles of drug substances*, 5, 61-85. doi:  
719 10.1016/S0099-5428(08)60315-9
- 720 Ihalainen, E., Lindström, L., & Mappes, J. (2007). Investigating Müllerian mimicry: predator  
721 learning and variation in prey defences. *Journal of Evolutionary Biology*, 20(2), 780-791.  
722 doi: 10.1111/j.1420-9101.2006.01234.x
- 723 Ihalainen, E., Lindström, L., Mappes, J., & Puolakkainen, S. (2008). Can experienced birds select  
724 for Müllerian mimicry? *Behavioral Ecology* 19, 362-368. doi: 10.1093/beheco/arm151
- 725 Ihalainen, E., Rowland, H. M., Speed, M. P., Ruxton, G. D., & Mappes, J. (2012). Prey community  
726 structure affects how predators select for Müllerian mimicry. [10.1098/rspb.2011.2360].  
727 *Proceedings of the Royal Society B: Biological Sciences*, 279(1736), 2099.
- 728 Ings, T. C., & Chittka, L. (2008). Speed-accuracy tradeoffs and false alarms in bee responses to  
729 cryptic predators. *Current Biology*, 18(19), 1520-1524. doi: 10.1016/j.cub.2008.07.074
- 730 Jiggins, C. D., & McMillan, W. O. (1997). The genetic basis of an adaptive radiation: warning  
731 colour in two *Heliconius* species. [10.1098/rspb.1997.0161]. *Proceedings of the Royal*  
732 *Society of London B*, 264(1385), 1167.
- 733 Joron, M., & Mallet, J. (1998). Diversity in mimicry: paradox or paradigm? . *Trends in Ecology &*  
734 *Evolution*, 13, 461-463.
- 735 Karlíková, Z., Veselý, P., Beránková, J., & Fuchs, R. (2016). Low ability of great tits to  
736 discriminate similarly inconspicuous edible and inedible prey. *Ethology*, 122(2), 180-191.  
737 doi: 10.1111/eth.12454
- 738 Kazemi, B., Gamberale-Stille, G., Tullberg, Birgitta S., & Leimar, O. (2014). Stimulus salience as  
739 an explanation for imperfect mimicry. *Current Biology*, 24(9), 965-969. doi:  
740 10.1016/j.cub.2014.02.061
- 741 Kazemi, B., Gamberale-Stille, G., & Leimar, O. (2015). Multi-trait mimicry and the relative  
742 salience of individual traits. *Proceedings of the Royal Society of London B*, 282(1818),

743 2015-2127. doi: 10.1098/rspb.2015.2127

744 Kikuchi, D. W., Mappes, J., Sherratt, T. N., & Valkonen, J. K. (2016). Selection for  
745 multicomponent mimicry: equal feature salience and variation in preferred traits. *Behavioral*  
746 *Ecology*, 27(5), 1515-1521. doi: 10.1093/beheco/arw072

747 Leimar, O., Enquist, M., & Sillen-Tullberg, B. (1986). Evolutionary stability of aposematic  
748 coloration and prey unprofitability: a theoretical analysis. *The American Naturalist*, 128(4),  
749 469-490. doi: 10.1086/284581

750 Lindstedt, C., Eager, H., Ihalainen, E., Kahilainen, A., Stevens, M., & Mappes, J. (2011). Direction  
751 and strength of selection by predators for the color of the aposematic wood tiger moth.  
752 *Behavioral Ecology*, 22(3), 580-587. doi: 10.1093/beheco/arr017

753 Lindstedt, C., Schroderus, E., Lindström, L., Mappes, T., & Mappes, J. (2016). Evolutionary  
754 constraints of warning signals: A genetic trade-off between the efficacy of larval and adult  
755 warning coloration can maintain variation in signal expression. *Evolution*, 70(11), 2562-  
756 2572. doi: 10.1111/evo.13066

757 Lindström, L., Alatalo, R. V., Lyytinen, A., & Mappes, J. (2001). Strong antiapostatic selection  
758 against novel rare aposematic prey. *Proceedings of the National Academy of Sciences of the*  
759 *United States of America*, 98(16), 9181-9184. doi: 10.1073/pnas.161071598

760 Lindström, L., Alatalo, R. V., & Mappes, J. (1997). Imperfect Batesian mimicry—the effects of the  
761 frequency and the distastefulness of the model. [10.1098/rspb.1997.0022]. *Proceedings of*  
762 *the Royal Society of London. Series B: Biological Sciences*, 264(1379), 149.

763 Lindström, L., Alatalo, R. V., & Mappes, J. (1999a). Reactions of hand-reared and wild-caught  
764 predators toward warningly colored, gregarious, and conspicuous prey. *Behavioral Ecology*,  
765 10(3), 317-322. doi: 10.1093/beheco/10.3.317

766 Lindström L., Alatalo R. V., Lyytinen, A., & Mappes, J. (2004). The effect of alternative prey on  
767 the dynamics of Batesian and Müllerian mimics. *Evolution*, 58, 1294-1302. doi:



768 10.1554/03-271.

769 Lindström, L., Alatalo, R. V., Mappes, J., Riipi, M., & Vertainen, L. (1999b). Can aposematic  
770 signals evolve by gradual change? *Nature*, 397(6716), 249-251. doi: 10.1038/16692

771 Lindström, L., Rowe, C., & Guilford, T. (2001). Pyrazine odour makes visually conspicuous prey  
772 aversive. *Proceedings of the Royal Society B: Biological Sciences*, 268(1463), 159-162.

773 Lynn, S. K. (2005). Learning to avoid aposematic prey. *Animal Behaviour*, 70, 1221-1226. doi:  
774 10.1016/j.anbehav.2005.03.010|ISSN 0003-3472

775 Mackintosh, N. J. (1975). A theory of attention: Variations in the associability of stimuli with  
776 reinforcement. *Psychological review*, 82(4), 276-298.

777 Mallet, J., & Barton, N. H. (1989). Strong natural selection in a warning-color hybrid zone.  
778 *Evolution*, 43(2), 421-431.

779 Mallet, J., & Joron, M. (1999). Evolution of diversity in warning color and mimicry:  
780 Polymorphisms, shifting balance, and speciation. *Annual Review of Ecology and*  
781 *Systematics*, 30, 201-233. doi: 10.1146/annurev.ecolsys.30.1.201

782 Mappes, J., & Alatalo R. V. (1997). Batesian mimicry and signal accuracy. *Evolution*, 51: 20148-  
783 2051. doi: 10.1111/j.1558-5646.1997.tb05129.x

784 Marples, N. M., & Kelly, D. J. (1999). Neophobia and dietary Conservatism: Two distinct  
785 processes? *Evolutionary Ecology*, 13(7-8), 641-653. doi: 10.1023/a:1011077731153

786 Marples, N. M., & Mappes, J. (2011). Can the dietary conservatism of predators compensate for  
787 positive frequency dependent selection against rare, conspicuous prey? *Evolutionary*  
788 *Ecology*, 25(4), 737-749.

789 Mettke-Hofmann, C., Winkler, H., & Leisler, B. (2002). The significance of ecological factors for  
790 exploration and neophobia in parrots. *Ethology*, 108(3), 249-272. doi: 10.1046/j.1439-  
791 0310.2002.00773.x

792 Müller, F. (1878). Ueber die Vortheile der Mimicry bei Schmetterlingen *Zoologischer Anzeiger*, 1,

793 54–55.

794 Nokelainen, O., Hegna, R. H., Reudler, J. H., Lindstedt, C., & Mappes, J. (2012). Trade-off  
795 between warning signal efficacy and mating success in the wood tiger moth. *Proceedings of*  
796 *the Royal Society B: Biological Sciences*, 279(1727), 257-265. doi: 10.1098/rspb.2011.0880

797 Nokelainen, O., Valkonen, J., Lindstedt, C., & Mappes, J. (2014). Changes in predator community  
798 structure shifts the efficacy of two warning signals in Arctiid moths. *Journal of Animal*  
799 *Ecology*, 83, 598-605. doi: 10.1111/1365-2656.12169

800 O'Donald, P., & Majerus, M. E. N. (1984). Polymorphism of melanic ladybirds maintained by  
801 frequency-dependent sexual selection *Biological Journal of the Linnean Society*, 23(2-3),  
802 101-111. doi: 10.1111/j.1095-8312.1984.tb00131.x

803 Pavlov, P. I. (1927). Conditioned reflexes: An investigation of the physiological activity of the  
804 cerebral cortex. *Annals of Neurosciences*, 17(3), 136.

805 Plowright, R. C., & Owen, R. E. (1980). The evolutionary significance of bumble bee color  
806 patterns: a mimetic interpretation. *Evolution*, 34(4), 622-637. doi: 10.2307/2408017

807 Poulton, E. B. (1890). *The Colours of Animals: Their Meaning and Use* (Vol. 26). London, U.K.:  
808 Kegan Paul, Trench, Trubner.

809 Prokopová, M., Veselý, P., Fuchs, R., & Zrzavý, J. (2010). The role of size and colour pattern in  
810 protection of developmental stages of the red firebug (*Pyrrhocoris apterus*) against avian  
811 predators. *Biological journal of the Linnean Society*, 100(4), 890-898. doi: 10.1111/j.1095-  
812 8312.2010.01463.x

813 Průchová, A., Nedvěd, O., Veselý, P., Ernestová, B., & Fuchs, R. (2014). Visual warning signals of  
814 the ladybird *Harmonia axyridis*: the avian predators' point of view. *Entomologia*  
815 *Experimentalis et Applicata*, 151(2), 128-134. doi: 10.1111/eth.12454

816 Riipi, M., Alatalo, R. V., Lindstrom, L., & Mappes, J. (2001). Multiple benefits of gregariousness  
817 cover detectability costs in aposematic aggregations. *Nature*, 413(6855), 512-514. doi:

- 818 10.1038/35097061
- 819 Rojas, B., & Endler, J. A. (2013). Sexual dimorphism and intra-populational colour pattern  
820 variation in the aposematic frog *Dendrobates tinctorius*. *Evolutionary Ecology*, 27(4), 739-  
821 753. doi: DOI 10.1007/s10682-013-9640-4
- 822 Rojas, B., Gordon, S. P., & Mappes, J. (2015). Frequency-dependent flight activity in the colour  
823 polymorphic wood tiger moth. *Current Zoology*, 61, 765-772.
- 824 Rojas, B., Burdfield-Steel, E. & Mappes, J. (2015). Primary vs. secondary defences in an  
825 aposematic moth: trade-off or additive effect? Submitted manuscript.
- 826 Rojas, B., Rautiala, P., & Mappes, J. (2014). Differential detectability of polymorphic warning  
827 signals under varying light environments *Behavioural Processes*, 109, 164-172. doi:  
828 10.1016/j.beproc.2014.08.014
- 829 Rojas, B., Burdfield-Steel, E., Pakkanen, H., Suisto, K., Maczka, M., Schulz, S., & Mappes, J.  
830 (2017). How to fight multiple enemies: target-specific chemical defences in an aposematic  
831 moth. *Proceedings of the Royal Society B*, 284, 20171424. doi: 10.1098/rspb.2017.1424
- 832 Rönkä, K., Mappes, J., Kaila, L., & Wahlberg, N. (2016). Putting *Parasemia* in its phylogenetic  
833 place: a molecular analysis of the subtribe Arctiina (Lepidoptera). *Systematic Entomology*,  
834 n/a-n/a. doi: 10.1111/syen.12194
- 835 Roper, T. J., & Redston, S. (1987). Conspicuousness of distasteful prey affects the strength and  
836 durability of one-trial avoidance learning. *Animal Behaviour*, 35(3), 739-747. doi:  
837 10.1016/S0003-3472(87)80110-0
- 838 Rothschild, M. (1961). Defensive odours and Müllerian mimicry among insects. *Transactions of the*  
839 *Royal Entomological Society of London*, 113(5), 101-123. doi: 10.1111/j.1365-  
840 2311.1961.tb00803.x
- 841 Rowe, C., & Guilford, T. (1996). Hidden colour aversions in domestic clicks triggered by pyrazine  
842 odours of insect warning displays. *Nature*, 383(6600), 520-522. doi: 10.1038/383520a0

843 Rowe, C., & Halpin, C. (2013). Why are warning displays multimodal? *Behavioral Ecology and*  
844 *Sociobiology*, 67(9), 1425-1439. doi: 10.1007/s00265-013-1515-8

845 Rowe, C., Lindström, L., & Lyytinen, A. (2004). The importance of pattern similarity between  
846 Mullerian mimics in predator avoidance learning. *Proceedings of the Royal Society B:*  
847 *Biological Sciences*, 271(1537), 407-413. doi: 10.1098/rspb.2003.2615

848 Rowland, H. M., Ihalainen, E., Lindström, L., Mappes, J., & Speed, M. P. (2007). Co-mimics have  
849 a mutualistic relationship despite unequal defences. *Nature*, 448, 64-67.

850 RStudio. (2015). RStudio: Integrated development environment for R (Version Version 0.99.441).  
851 Available from <http://www.rstudio.org/>.

852 Ruxton, G. D., Franks, D. W., Balogh, A. C. V., & Leimar, O. (2008). Evolutionary implications of  
853 the form of predator generalization for aposematic signals and mimicry in prey *Evolution*,  
854 62(11), 2913-2921. doi: 10.1111/j.1558-5646.2008.00485.x

855 Ruxton, G. D., Sherratt, T. N., & Speed, M. P. (2004). *Avoiding Attack: the evolutionary ecology of*  
856 *crypsis, warning signals and mimicry*. Oxford, U.K.: Oxford University Press.

857 Sherratt, T. N. (2002). The evolution of imperfect mimicry. *Behavioral Ecology*, 13(6), 821-826.  
858 doi: 10.1093/beheco/13.6.821

859 Sherratt, T. N., & Beatty, C. D. (2003). The evolution of warning signals as reliable indicators of  
860 prey defense. *American Naturalist*, 162(4), 377-389.

861 Sherratt, T. N., & Macdougall, A. D. (1995). Some population consequences of variation in  
862 preference among individual predators. *Biological Journal of the Linnean Society*, 55(2),  
863 93-107. doi: 10.1111/j.1095-8312.1995.tb01053.x

864 Siddall, E. C., & Marples, N. M. (2008). Better to be bimodal: the interaction of color and odor on  
865 learning and memory. *Behavioral Ecology*, 19(2), 425-432. doi: 10.1093/beheco/arm155

866 Sillén-Tullberg, B. (1985). The significance of coloration per se, independent of background, for  
867 predator avoidance of aposematic prey. *Animal Behaviour*, 33(4), 1382-1384. doi:

868 10.1016/S0003-3472(85)80211-6

869 Skelhorn, J., Halpin, C. G., & Rowe, C. (2016). Learning about aposematic prey. *Behavioral*  
870 *Ecology*, 27(4), 955-964. doi: 10.1093/beheco/arw009

871 Skelhorn, J., & Rowe, C. (2007). Predators' toxin burdens influence their strategic decisions to eat  
872 toxic prey. *Current Biology*, 17(17), 1479-1483. doi: 10.1016/j.cub.2007.07.064

873 Speed, M. P. (2000). Warning signals, receiver psychology and predator memory. *Animal*  
874 *Behaviour*, 60, 269-278. doi: 10.1006/anbe.2000.1430

875 Svádová, K., Exnerová, A., Stys, P., Landová, E., Valenta, J., Fucíková, A., & Socha, R. (2009).  
876 Role of different colours of aposematic insects in learning, memory and generalization of  
877 naive bird predators. *Animal Behaviour*, 77(2), 327-336. doi:  
878 10.1016/j.anbehav.2008.09.034

879 ten Cate, C., & Rowe, C. (2007). Biases in signal evolution: learning makes a difference. *Trends in*  
880 *Ecology & Evolution*, 22(7), 380-387.

881 Terhune, E. C. (1977). Components of a visual stimulus used by scrub jays to discriminate a  
882 Batesian model. *The American Naturalist*, 111(979), 435-451. doi: 10.1086/283178

883 Therneau, T. M. (2015). coxme: Mixed Effects Cox Models (Version 2.2-5). [https://CRAN.R-](https://CRAN.R-project.org/package=coxme)  
884 [project.org/package=coxme](https://CRAN.R-project.org/package=coxme)

885 Trimmer, P. C., Houston, A. I., Marshall, J. A. R., Mendl, M. T., Paul, E. S., & McNamara, J. M.  
886 (2011). Decision-making under uncertainty: biases and Bayesians. *Animal Cognition*, 14(4),  
887 465-476. doi: 10.1007/s10071-011-0387-4

888 Turini, A., Veselý, P., & Fuchs, R. (2016). Five species of passerine bird differ in their ability to  
889 detect Batesian mimics. *Biological journal of the Linnean Society*, 117(4), 832-841. doi:  
890 10.1111/bij.12708

891 Veselý, P., Veselá, S., Fuchs, R., & Zrzavý, J. (2006). Are gregarious red-black shieldbugs,  
892 *Graphosoma lineatum* (Hemiptera: Pentatomidae), really aposematic? An experimental

- 893 approach. *Evolutionary Ecology Research*, 8(5), 881-890.
- 894 Veselý, P., & Fuchs, R. (2009). Newly emerged Batesian mimicry protects only unfamiliar prey.  
895 *Evolutionary Ecology*, 23(6), 919. doi: 10.1007/s10682-008-9281-1
- 896 Veselý, P., Luhanová, D., Prášková, M., & Fuchs, R. (2013). Generalization of mimics imperfect in  
897 colour patterns: the point of view of wild avian predators. *Ethology*, 119(2), 138-145. doi:  
898 10.1111/eth.12045
- 899 Veselý, P., Ernestová, B., Nedvěd, O., & Fuchs, R. (2017). Do predator energy demands or  
900 previous exposure influence protection by aposematic coloration of prey? *Current Zoology*,  
901 63(3), 259-267. doi: 10.1093/cz/zow057
- 902 Waldron, S. J., Endler, J. A., Valkonen, J. K., Honma, A., Dobler, S., & Mappes, J. (2017).  
903 Experimental evidence suggests that specular reflectance and glossy appearance help  
904 amplify warning signals. *Scientific Reports*, 7, 257. doi: 10.1038/s41598-017-00217-5
- 905 Webster, S. J., & Lefebvre, L. (2000). Neophobia by the Lesser-Antillean Bullfinch, a foraging  
906 generalist, and the Bananaquit, a nectar specialist. *The Wilson Bulletin*, 112(3), 424-427.  
907 doi: 10.1676/0043-5643(2000)112[0424:NBTLAB]2.0.CO;2

908

909 Appendix

910

911 **Table 1.** Distribution of colours chosen first in the preference test trials (phase 1)

	White first	Yellow first	Red first	<i>N</i>	<i>df</i>	$\chi^2$	<i>P</i>
Trial 1	34	32	34	53	2	0.04	0.98
Trial 2	44	22%	34	41	2	2.98	0.23
Trial 3	29	27%	44	41	2	2.10	0.35

912

913 The percentages of birds that chose white, yellow or red models first in the three trials of  
 914 the preference test, and the corresponding chi-square comparison for preference for each  
 915 trial, are shown.

916

917

918 **Table 2.** Proportions ( $\pm$  SE) of tested colour morphs attacked in the generalization test  
919 trials (phase 3) in relation to the colour learned

---

Colour learned	Colour tested	First trial	Second trial	Third trial
White	Yellow	0.85 $\pm$ 0.09	0.92 $\pm$ 0.07	0.64 $\pm$ 0.13
White	Red	0.85 $\pm$ 0.09	0.78 $\pm$ 0.11	0.78 $\pm$ 0.11
Yellow	White	0.73 $\pm$ 0.11	0.73 $\pm$ 0.11	0.73 $\pm$ 0.11
Yellow	Red	0.86 $\pm$ 0.09	0.73 $\pm$ 0.11	0.60 $\pm$ 0.13
Red	White	0.93 $\pm$ 0.06	0.86 $\pm$ 0.09	0.73 $\pm$ 0.11
Red	Yellow	0.93 $\pm$ 0.06	0.86 $\pm$ 0.09	0.86 $\pm$ 0.09

---

920



921 **Table A1.** Test for asymmetric generalization in the first generalization trial

Model	<i>df</i>	LRT	Pr(Chi)	Model AIC
<b>(Intercept) + colour combination</b>	5	10.70	0.058	37.4
(Intercept)				38.1

  

Random effects	Variance	SD		
Bird ID	4808	69.34		

  

Fixed effects	Estimate	SE	Z	P
(Intercept: colour combination: yw)	12.48	3.32	3.75	<0.001
Colour combination: yr	14.85	5.22	2.85	0.0044
Colour combination: rw	2.27	8.04	0.28	0.78
Colour combination: ry	2.27	8.19	0.28	0.78
Colour combination: wr	1.38	6.16	0.22	0.82
Colour combination: wy	1.38	6.15	0.22	0.82

923 LRT: likelihood ratio test; y: yellow; w: white; r: red. Model selection was based on model  
 924 fit, i.e. the model chosen was the one with the lowest Akaike information criterion (AIC)

925 value. The significance level of  $\chi^2$  (Chi) indicates a change from the model with colour  
926 combination as an explanatory variable to the model below, with intercept only. Estimates  
927 of the best-fitting model (in bold) are shown below. Of the colour combinations, the  
928 combination of yellow learned and white offered had least attacks, and was thus set to the  
929 intercept.

930

931

932 **Table A2.** Test for asymmetric generalization in all three generalization trials

Model	<i>df</i>	LRT	Pr(Chi)	model AIC
(Intercept) + colour combination	5	4.32	0.50	161.9
<b>(Intercept)</b>				156.2

933

Random effects	Variance	SD
Trial: Bird ID	167.82	12.96
Bird ID	58.01	7.62

Fixed effects	Estimate	SE	Z	P
(Intercept)	10.05	1.57	6.42	< 0.001

934 LRT: likelihood ratio test. Model selection was based on model fit, i.e. the model chosen  
 935 was the one with the lowest Akaike information criterion (AIC) value. The significance level  
 936 of  $\chi^2$  (Chi) indicates a change from the model with colour combination as an explanatory  
 937 variable to the model below, with intercept only. Estimates of the best-fitting model (in  
 938 bold) are shown below.

939

940

941 **Table A3.** Comparisons of colours attacked first in the generalization trials

	Colour learned	Colour tested	Attacked first	<i>df</i>	$\chi^2$	<i>P</i>	
First trial	White	Yellow	6/14	1	0	1	
		Red	6/14				
	Yellow	White	6/15	1	0	1	
		Red	7/15				
	Three trials pooled	Red	White	8/15	1	0.13	0.71
			Yellow	6/15			
White		Yellow	12/42	1	5.89	<b>0.02</b>	
		Red	24/42				
Three trials pooled	Yellow	White	20/45	1	0.75	0.39	
		Red	15/45				
	Red	White	22/45	1	0.41	0.52	
	Yellow	18/45					

942 Chi-square comparisons of how many times each tested colour morph was attacked first in  
 943 the generalization trials, by the colour morph learned. Models that were not  
 944 attacked or attacked second are included in the total number of models offered.  
 945 Bold indicates significant difference.

946 Figure captions

947 **Figure 1.** (a) Typical wood tiger moth colour morphs from central Finland (leg. Kari  
948 Kulmala), (b) artificial white, yellow and red moth wings used in the experiment and (c)  
949 reflectance curves of the white, yellow and red hindwings of real moths (darker colours)  
950 compared to reflectance curves from white, yellow and red model hindwings (lighter  
951 colours). Spectral measurements were taken from three wild-caught individuals of each  
952 colour from the spots marked with blue circles on the white moth. The same spots were  
953 used to measure the model hindwing colours. Model wings were set in a more natural  
954 posture, less spread than the spread collection samples, but unfolded to show the  
955 hindwing colour.

956

957 **Figure 2.** Schematic illustration of the experimental design. Each green circle represents a  
958 platform presented to a bird in one trial. Moth models as presented to a bird that learned to  
959 avoid the red morph are illustrated on the platforms and alternative food offered between  
960 the trials is shown above the platforms. For details of the experimental protocol see  
961 Methods (Experimental procedures; phases 1-3).

962

963 **Figure 3.** Proportion of models attacked during the learning trials for each colour model.  
964 The lines represent the cumulative attacks on unpalatable moth models of white (black  
965 line), yellow (yellow line) or red (red line) hindwing colour.

966

967 **Figure 4.** Proportions ( $\pm$  SE) of models that were attacked in the first trial of the  
968 generalization test. Symbol styles refer to white (black circle), yellow (yellow square) and  
969 red (red triangle) models offered in the test.