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**Predator selection on multicomponent warning signals in an
aposematic moth**

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25 **ABSTRACT**

26 Aposematic prey advertise their unprofitability with conspicuous warning signals that are
27 often composed of multiple color patterns. Many species show intraspecific variation in
28 these patterns even though selection is expected to favor invariable warning signals that
29 enhance predator learning. However, if predators acquire avoidance to specific signal
30 components, this might relax selection on other aposematic traits and explain variability.
31 Here we investigated this idea in the aposematic moth *Amata nigriceps* that has
32 conspicuous black and orange coloration. The size of the orange spots in the wings is
33 highly variable between individuals, whereas the number and width of orange abdominal
34 stripes remains consistent. We produced artificial moths that varied in the proportion of
35 orange in the wings or the presence of abdominal stripes. We presented these to a natural
36 avian predator, the noisy miner (*Manorina melanocephala*), and recorded how different
37 warning signal components influenced their attack decisions. When moth models had
38 orange stripes on the abdomen, birds did not discriminate between different wing signals.
39 However, when the stripes on the abdomen were removed, birds chose the model with
40 smaller wing spots. In addition, we found that birds were more likely to attack moths with
41 a smaller number of abdominal stripes. Together, our results suggest that bird predators
42 primarily pay attention to the abdominal stripes of *A. nigriceps*, and this could relax
43 selection on wing coloration. Our study highlights the importance of considering
44 individual warning signal components if we are to understand how predation shapes
45 selection on prey warning coloration.

46 **Keywords:** aposematism, color pattern, Lepidoptera, noisy miner, salience, warning
47 signals

48

49

50 INTRODUCTION

51 Many aposematic prey use visual warning signals, typically conspicuous coloration, to
52 warn predators about their unprofitability, such as toxic or unpalatable chemical defences
53 (Poulton 1890). Predators need to learn to recognise warning signals, and conspicuous
54 and consistent signals enhance this avoidance learning (Gittleman and Harvey 1980;
55 Roper and Redston 1987). Selection by predators is therefore expected to lead to
56 invariable warning signals, but many aposematic species show considerable intraspecific
57 variation in their color patterns (Briolat et al. 2019). This is often explained by limits to
58 optimal warning signal expression, including costs associated with signal production
59 (Blount et al. 2012), and trade-offs with other functions of coloration, such as sexual
60 selection (Maan and Cummings, 2008) or thermoregulation (Lindstedt et al. 2009; Hegna
61 et al. 2013). However, selection pressures from predators might also be more complex
62 than traditionally assumed (Endler and Mappes 2004).

63 There is both within and between species variation in predator responses to
64 aposematic prey (Endler and Mappes 2004), and spatial and temporal variation in predator
65 pressure can lead to variable warning signals in prey (Nokelainen et al. 2014; Rönkä et
66 al. 2020). Predator species may, for example, differ in their visual (Mochida 2011) and
67 cognitive abilities (Rowland et al. 2017), or in their resistance to prey toxins (Fink et al.
68 1983; Brodie and Brodie 1990). In addition, individuals of the same predator species
69 differ in their prior experience (Exnerová et al. 2007), personality (Exnerová et al. 2010),
70 and current physiological condition (Barnett et al. 2007; Skelhorn and Rowe 2007), which
71 can influence their decision to attack aposematic prey. This heterogeneity among
72 predators can generate variation in selection pressure for signal conspicuousness and help
73 maintain variable color patterns in aposematic species (Endler and Mappes 2004;
74 Nokelainen et al. 2014; Rönkä et al. 2020).

Besides predator diversity, the avoidance learning process plays an important role in the evolution of warning signals (Skelhorn et al. 2016). Warning signals are often complex, and different signal components may elicit distinct predator responses, which might heighten selection on some elements, while relaxing selection on others (Winters et al. 2017). For example, many warning signals are multimodal, consisting of visual signals, sounds, odours, and chemical secretions that may have interactive effects (Rowe and Halpin 2013). Another potential factor influencing warning signal efficacy is prey shape or posture that might be particularly important when visual signals are combined with deimatic behavior (Hernández-Palma et al. 2023; Riley et al. 2023). Even within one modality, there can be multicomponent signals that consist of different elements, such as visual warning signals that are composed of distinct colors, shapes and patterns on different body regions (Rowe 1999; Bradbury and Vehrencamp 2011). Predators might use several components when making foraging decisions (Pegram et al. 2013; Kikuchi et al. 2016), or alternatively base prey avoidance on a specific component, which could allow variation to exist in other color patterns (Winters et al. 2017). For instance, fish predators associate the yellow rim of nudibranchs with their unpalatability but do not learn to avoid nudibranchs based on their red spots, which might lead to relaxed selection on consistency of red-spotted patterns (Winters et al. 2017). Indeed, red spots were found to vary within and between nudibranch populations, whereas the yellow rim remained invariable (Winters et al. 2017). Considering the impact of individual color pattern elements on predator behavior may therefore help us to understand the maintenance of unexpected warning signal variation in aposematic species.

Here, we investigated how predators respond to color pattern elements of aposematic moths, *Amata nigriceps*, that are found along the east coast of Australia. The moths are chemically defended (Rothschild et al. 1984) and have black and orange color patterns on their wings and body. The wing patterns include orange spots against a black

101 background, and the body coloration consists of orange and black stripes on the abdomen
102 (Figure 1). Both wing spots and abdominal stripes could function as a warning signal, but
103 their relative importance on predator attack decisions has not yet been tested. The two
104 color patterns also differ in visibility: when the moths are resting, the wings can cover a
105 large proportion of the abdomen, making the stripes invisible. The size of the orange spots
106 on the wings is highly heritable and varies from 10 to 30% of the wing area within and
107 across populations, and females typically have larger orange spots than males (Binns et
108 al. 2022). Orange stripes on the abdomen, in contrast, are more consistent, with each sex
109 having a fixed number of stripes in abdominal segments (females: five orange stripes;
110 males: six orange stripes) and low variation in stripe width (Binns et al. in prep.). What
111 role predation plays in the maintenance of this variation in the wings and consistency in
112 abdominal stripes among individuals, however, remains untested.

113 We conducted predation trials in the field to test how orange wing spots and
114 abdominal stripes influence predator foraging decisions. We used artificial moth models
115 and noisy miners (*Manorina melanoccephala*) as predators. Predation attempts on *A.*
116 *nigriceps* are difficult to observe in the wild and their main predators are therefore
117 unknown, but noisy miners are generalist feeders that regularly incorporate moths into
118 their diet (Higgins et al. 2006). The experiments were conducted during the *A. nigriceps*
119 flight season in locations where both *A. nigriceps* and noisy miners are commonly found,
120 so birds were likely to have encountered the moths before the trials. We conducted three
121 different experiments where birds were presented with two-choice preference tests. In
122 Experiment 1, our aim was to investigate whether birds discriminate between moths with
123 small and large wing spots when the effect of abdominal stripes was removed by painting
124 the model abdomens black. In Experiment 2, we tested biases towards the same small vs.
125 large wing spots but this time the model abdomens included orange stripes to investigate
126 if this changed how birds perceived the wing signals. In Experiment 3, both moth models

127 had the same size wing spots, but we manipulated the number of abdominal stripes to
128 investigate their effect on predator foraging decisions.

129

130 **METHODS**

131 **Predator species and locations**

132 We conducted field experiments with noisy miners between September 2020 and March
133 2022 with the permission from the Animal Ethics Committees at Macquarie University
134 (ARA 2020/009) and at the University of New England (AEC20-099). Experiment 1 was
135 conducted in two field sites: Macquarie University campus, Sydney, NSW
136 (Wallumattagal Land, 33°46'26" S 151°06'46" E), and Newholme Research Station of
137 the University of New England, Armidale, NSW (Anēwan Land, 30°25'26" S 151°39'13"
138 E). Experiments 2 and 3 were conducted only on Macquarie University campus. The
139 noisy miner is a medium-sized honeyeater endemic to southeastern Australia (Higgins et
140 al. 2006). The species is a generalist forager that feeds on nectar, fruits and insects. The
141 breeding season occurs from June to December and can include several broods (Higgins
142 et al. 2006). Noisy miners are inquisitive, and readily explore new objects and become
143 habituated to humans quickly in urban areas. They live in large colonies that have
144 complex social structures, and individuals in the colony are usually found in the same
145 geographical areas (Dow 1979). The mean diameter of these 'activity spaces' averages
146 114m for males and 74m for females (Dow 1979). To minimise the likelihood of testing
147 the same individuals several times, we chose test locations that were at least 500m apart
148 on Macquarie University campus. The test locations at Newholme Research Station were
149 closer to each other (approximately 250m), but there the majority of the birds were color
150 banded and we could individually identify birds to ensure that the same individual did not
151 visit multiple locations.

152 **Artificial moths**

153 ***Wings***

154 For all three experiments we used the same methods to make the wings. We created
155 artificial moths with ‘small’ and ‘large’ orange wing spots that represented the observed
156 variation in warning signal size among 200 *A. nigriceps* collected from Sydney, Australia,
157 between years 2018-2019 (Binns et al. in prep.). We designed signals to match the lower
158 and upper quartiles of this variation, resulting in 15.5% of the wing area orange in the
159 small and 22.1% of the wing area orange in the large spot (Figure 2a). Similar variation
160 in wing signals is observed in the moth population on Macquarie University campus
161 (Binns et al. 2022) and we expect to find both signal types also in our other study location
162 at Newholme Research Station, although this has not been quantified. The model wings
163 were created from images of real *A. nigriceps* moths that represented small (15.5%
164 orange) and large (22.1% orange) wing spots, using Adobe After Effects CS4
165 (Christiansen 2013). The total number of spots was held constant at 22. We used average
166 wing length (15mm; Binns et al. in prep.), and the same wing shape for both signals (see
167 Supplementary Material for details of model preparation). The finished wings were
168 duplicated and horizontally flipped to obtain symmetric left and right wings. These were
169 printed on Kodak matte photo paper, using an Epson Stylus Photo RE3000 printer and
170 Genuine Epson 157 ink. To ensure that orange wing spots matched the real color of *A.*
171 *nigriceps* moths as closely as possible, we chose the orange color based on the color
172 reflectance values of orange wing spots of real *A. nigriceps* moths (Figure S1a in
173 Supplementary Material).

174 ***Bodies***

175 We made the model abdomens differently to suit the questions for each of our three
176 experiments. In Experiment 1, abdomens were left plain black to isolate the effect of the

177 wing spots. Our pilot studies suggested that birds would not attack moth models with clay
178 bodies. We therefore increased birds' motivation to attack models by making moth bodies
179 of cake (Woolworths Madeira Cake) that has been used successfully as a palatable reward
180 in previous studies with noisy miners (Farrow et al. 2017), and by pre-training the birds
181 to visit feeders containing cake (see below). To make the bodies black, we mixed 10g of
182 cake with 2mL of black food dye (Queen Classic Black Food Color). We then used this
183 mixture to prepare 12mm long and 3mm wide bodies (weight $0.3\text{g} \pm 0.05\text{g}$) that
184 resembled the real size of *A. nigriceps* moths (Binns et al. in prep.). These were placed
185 between the paper wings.

186 In Experiments 2 and 3, we tested how birds responded to moth models when body
187 coloration resembled the real coloration of *A. nigriceps* (Figure 1). This included adding
188 an orange triangle to the thorax, five orange stripes to the abdomen and one orange stripe
189 behind the head, as well as orange tip to the abdomen (Figure 2a). For these two
190 experiments, the bodies were made using Monster Clay® medium modeling clay (The
191 Monster Makers, Ohio, USA) as it was not possible to paint orange markings on cake.
192 The clay was heated up to a liquid, poured into 12mm long and 3mm wide molds and
193 allowed to set for 24hrs (Binns et al. in prep.). The bodies were then painted black and
194 orange using MontMarte® Acrylic paints, using a mixture of paints closest to the orange
195 stripes of the real moths, based on the color reflectance measurements (see Figure S1b in
196 Supplementary Material). In Experiment 2, all model bodies included five stripes on the
197 abdomen. For Experiment 3, we painted half of the bodies with only three abdominal
198 stripes and half of the bodies with five abdominal stripes (Figure 2a) to test whether birds
199 use the number of stripes as a cue in their foraging decisions. The width of the stripes was
200 the same in bodies with three and five stripes and was based on the measurements from
201 real moths (0.5 mm, Binns et al. in prep.). The painted bodies were glued to the paper
202 wings using a non-toxic UHU glue stick. Because we found that birds did not attack these

203 clay bodies, a piece of cake (similar shape and size used in the Experiment 1 but not
204 colored black) was placed under the moth models to encourage birds to choose between
205 the two options. Even though there was a slight change in the experimental design
206 between Experiment 1 and Experiments 2 and 3 (cake being between the wings or under
207 the clay body), we found that birds paid attention to the signal elements in both cases (see
208 results), and this methodological difference was therefore unlikely to influence our
209 results.

210 **Pre-training and experimental setup**

211 Before the experimental predation trials, birds were trained to visit round feeding trays
212 (17cm diameter) that were surrounded by wire mesh with one opening where the birds
213 could enter (Figure 2b). This ensured that only one bird at a time could visit the feeder,
214 and that birds always approached the moths from the same direction. We used four
215 feeding trays that were attached to two feeding poles (approximately 80cm from each
216 other; Figure 2b), so that several birds could be trained and tested simultaneously. We
217 started to train the birds to visit the feeders approximately 10 days before the experiment
218 by offering them pieces of cake until birds were habituated to the feeders and approached
219 them immediately when the food was presented (approximately after five days of
220 training). After the birds were trained to the feeders, they were further trained on stimuli
221 relevant to Experiment 1, 2, or 3. Depending on the experiment for which the birds were
222 being trained, birds learned either to eat cake pieces that were colored black with food
223 dye (Experiment 1), or to find cake pieces placed under a piece of brown paper that was
224 similar shape to the moths used in the experimental trials (Experiments 2 and 3). The
225 birds were deemed ‘trained’ when they readily consumed the black cake or had learned
226 to find the cake from under the brown paper. This took approximately five days to achieve
227 and at the end of the training birds typically flew to the feeders immediately after they
228 were presented and ate the cake without hesitation.

229 **Predation trials**

230 We conducted three different predation experiments with artificial *A. nigriceps* moths to
231 investigate predator responses to Experiment 1) small vs. large orange wing spots with
232 black abdomen, Experiment 2) small vs. large orange wing spots with striped abdomen,
233 and Experiment 3) low vs. high number of abdominal stripes with wing spots held
234 constant (small spots, Figure 2a). In each experiment, we recorded the first choice of the
235 birds (which cake piece was eaten first). The latency to attack the models was not
236 analysed because it was not possible to tell from the videos the exact time when birds saw
237 the models, and both signal types were not necessarily attacked during the same visit to
238 the feeder (both models attacked: $n = 47$, only one model attacked: $n = 38$).

239 ***Experiment 1: The effect of wing spots without abdominal stripes***

240 The first experiment was conducted on Macquarie University campus from September
241 2020 to February 2021, and at Newholme Research Station in April 2021. At each field
242 site, we conducted foraging trials in eight different locations. In the trials, a pair of moth
243 models with small and large spots were presented to birds on four feeding trays (Figure
244 2b). The paper models were glued to a green background (green paper attached to the
245 tray) 3.5cm apart from each other, randomizing their side in each trial, and the black cake
246 body was added between the wings.

247 The birds were observed from a distance (approximately 5m), and their choices
248 were recorded using small video cameras (Action Camera, Muson 4K) attached to the
249 feeding poles (Figure 2b). We recorded the birds' choice when they encountered the
250 moths for the first time, because subsequent contact could lead the bird to learn that the
251 cake between the moth wings is palatable regardless of the signal size and change their
252 response to the signals. As the birds on Macquarie University campus were not
253 individually identifiable, we could not distinguish different individuals that were visiting

254 the feeders. To minimise the likelihood of testing the same individuals twice, we only
255 used the data from birds that arrived first in each test location (1-4 birds per location,
256 depending on how easy it was to follow and distinguish them from each other). At
257 Newholme Research Station, nine of the birds that visited the experimental setup were
258 color-banded, so we could identify them from the video recordings. Birds that were not
259 banded ($n = 6$) were included in the data only if they were the first 1-4 birds per location
260 (following the same protocol as on Macquarie University campus). The feeders were
261 recorded for 30 min on Macquarie University campus and for 60 min at Newholme
262 Research Station, because at Newholme the color bands enabled us to identify individuals
263 that arrived at the feeders later. The moth bodies were replaced each time birds visited
264 the feeders, so that birds always had a choice between the two signals. We recorded the
265 first choices of 32 birds that we could confidently identify as different individuals
266 (Macquarie University campus: $n = 17$, Newholme Research Station: $n = 15$).

267 ***Experiment 2: The effect of wing spots and abdominal stripes***

268 The second experiment was conducted in 10 different locations on Macquarie University
269 campus from October to December 2021. Seven of these locations were the same as in
270 Experiment 1, and it is therefore likely that some birds participated in both experiments,
271 which could have influenced their responses. However, birds were exposed to the models
272 for only 30 minutes, and there were eight months between the two experiments, so the
273 groups were likely to include new individuals and any ‘repeat birds’ did not have recent
274 experience of the models. We used the same wing patterns as in Experiment 1, but this
275 time the moth bodies were made of clay and featured abdominal stripes (Figure 2a). We
276 followed the same methods as in Experiment 1, but the moth models were glued to the
277 background so that the clay body was lifted up slightly and a piece of cake could be placed
278 under it. To qualify as a first choice, birds had to eat the cake under the model. We

279 followed the protocol from Experiment 1 to record the first choices of birds that we could
280 identify as different individuals, which resulted in a sample size of 27 birds.

281 ***Experiment 3: The effect of abdominal stripes***

282 The third experiment was conducted on Macquarie University campus from February to
283 March 2022. We used the same 10 test locations as in Experiment 2, so most birds were
284 likely to have experienced the models before, with the addition of young from the 2021-
285 2022 breeding season. For those birds that had experienced the models before, the most
286 recent exposure to the models was at least three months prior to Experiment 3. We offered
287 birds two moth models that had the same small orange wing spots, but that differed in the
288 number of abdominal stripes, having either five or three stripes (Figure 2a). We followed
289 the protocol from Experiment 2, placing a piece of cake under the moth models and
290 recording which one was eaten first. As in previous experiments, we only used the first
291 choices of the birds that we were confident to be different individuals ($n = 26$).

292 **Statistical analyses**

293 We investigated whether birds had preferences towards the different signal types using
294 generalized linear models with a binomial error distribution. The order in which the
295 models were attacked (which cake was eaten first) was used as the response variable and
296 this was explained by the signal type (Experiments 1 and 2: size of wing spot, small and
297 large; Experiment 3: number of stripes, low and high); and the side of the tray (left and
298 right). Because the trials in Experiment 1 were conducted at two sites (Macquarie
299 University campus and Newholme Research Station), we also included an interaction
300 between site and signal type to test for any differences in bird responses between the sites.
301 Non-significant interaction was removed from the final model (see results), but both main
302 effects (signal type and side of tray) were retained in the models regardless of their

significance. All analyses were conducted using R version 3.6.1 (R Core team 2019). The graphs were made using the package ggplot2 (Wickham 2016).

RESULTS

Experiment 1: The effect of wing spots without abdominal stripes

The choices of the birds were similar in both test sites (signal choice \times test site: estimate = 0.791 ± 1.096 , $Z = 0.721$, $P = 0.47$), so we removed ‘test site’ from the final model. We found that birds were more likely to attack a moth model with small wing spots as their first choice, compared to a moth with large wing spots (estimate = -1.609 ± 0.548 , $Z = -2.938$, $P = 0.003$; Figure 3a). There were no biases towards prey on the left or right side of the tray (estimate = 0.223 ± 0.548 , $Z = 0.407$, $P = 0.68$).

Experiment 2: The effect of wing spots and abdominal stripes

When presented with moth models that included orange abdominal stripes, birds no longer had a significant preference for small wing spots (estimate = 0.406 ± 0.553 , $Z = 0.734$, $P = 0.46$; Figure 3b). Similar to the first experiment, the side of the tray did not influence the initial attack choices (estimate = 0.406 ± 0.553 , $Z = 0.734$, $P = 0.46$).

Experiment 3: The effect of abdominal stripes

There was a significant effect of abdominal stripes on bird choices, with a higher number of birds attacking the moth model that had only three orange stripes on the abdomen (compared to moths with five stripes: estimate = -1.386 ± 0.607 , $Z = -2.285$, $P = 0.022$; Figure 3c). Again, there were no biases towards prey on the left or right side of the tray (estimate = 0.575 ± 0.607 , $Z = 0.948$, $P = 0.34$).

DISCUSSION

327 Aposematic animals often have multicomponent warning signals, and understanding the
328 selection pressures that maintain warning coloration requires exploring the relative
329 importance of each component on predator foraging decisions (Winters et al. 2017). Here,
330 we investigated how avian predators respond to two warning signal components of *A.*
331 *nigriceps* moths: orange wing spots and orange stripes on the abdomen. Wings with small
332 spots were more often attacked first by predators compared to those with large spots, but
333 only when orange stripes on the abdomen were not visible. The number of orange stripes
334 on the abdomen influenced predators' foraging decisions, with birds being more likely to
335 attack a moth model with a smaller number of stripes. Our results therefore suggest that
336 orange stripes in *A. nigriceps* are an important warning signal component and a primary
337 cue for predators whereas wing spots are used only when abdominal signals are not
338 available. This could reduce selection on consistency in wing patterns, and as such our
339 study provides further support for the idea that warning signal variation can be explained
340 by differential selection pressures on individual color pattern elements (Winters et al.
341 2017).

342 Different components of aposematic signals vary in their importance in
343 discrimination learning and generalization, and predators often base their foraging
344 decisions on high-salience traits compared to less salient ones (Bain et al. 2007; Kazemi
345 et al. 2014; Kikuchi and Sherratt 2015; Sherratt et al. 2015). For example, color is
346 typically found to be a more important cue for predators than patterns or prey shape
347 (Gamberale-Stille and Guilford 2003; Aronsson and Gamberale-Stille 2008; Kazemi et
348 al. 2014; Sherratt et al. 2015; Riley et al. 2023; but see Lee et al. 2018; Linke et al. 2022).
349 Similarly, different color pattern elements may differ in their salience, and predators can
350 associate prey defence with one specific color pattern (Kikuchi et al. 2016; Winters et al.
351 2017). This seemed to be the case in our study where abdominal stripes overshadowed
352 the effect of wing spots, suggesting that the stripes are a primary cue for predators. This

353 result might have been influenced by our study design where birds consumed only the
354 moth body (or a cake under it), which could have directed their attention to the abdomen
355 coloration, but this is unlikely because bird did base their attack decisions on the wing
356 signal in Experiment 1. Furthermore, because predation attempts on *A. nigriceps* are
357 difficult to observe in the wild, we do not know where birds usually target their attacks,
358 and which parts of the moths (if any) are consumed. Since we conducted our experiments
359 in the field, the previous experience of the specific noisy miners involved is also
360 unknown. However, the timing of the different experiments overlapped, so the number of
361 less experienced juveniles was likely to be similar in each experiment. In addition, we
362 conducted all the experiments during the *A. nigriceps* flight season and the moths
363 commonly occur in the study areas, so we assume that most birds had encountered them
364 previously.

365 Alternatively, it is possible that predators make their attack decisions based on the
366 overall conspicuousness of the prey (Dreher et al. 2015), and specific color pattern
367 elements are less important. This might provide another explanation for our results
368 because adding orange abdominal stripes reduced the difference in conspicuousness
369 between the two wing signals we tested, and could therefore explain why birds did not
370 discriminate between them. It is also possible that after reaching some threshold value in
371 the overall proportion of orange, slight variation in wing coloration no longer affects
372 predator foraging decisions, perhaps because the prey is perceived to be too toxic to
373 attack. In some species, more conspicuous warning signals are associated with higher
374 toxicity (i.e., honest signalling, Summers et al. 2015), which could explain predator
375 decisions to choose a less conspicuous alternative. However, there is no evidence of an
376 association between wing coloration and toxicity in *A. nigriceps* (Hämäläinen et al. in
377 prep.). Similarly, there is no evidence of toxicity differences between the sexes (Binns et
378 al. in prep.), even though females have larger orange wing signals than males (Binns et

al. 2022). Females and males also differ in the abdominal patterns, with males having one more orange stripe (six stripes) than females (five stripes), but this does not influence the proportion of orange on the abdomen that is similar in both sexes (Binns et al. in prep.). Because our aim was to test how the amount of orange in each color pattern influences predator responses, we manipulated both the number of stripes and the proportion of orange on the abdomen, and it is not possible to disentangle these two effects. Testing the effects of the pattern and overall conspicuousness separately therefore provides a prospective area for future research.

Although orange abdominal stripes seemed to be a primary cue to predators, we also found that the wing spots were important when the stripes were not visible. This leads to the question – how visible is each warning signal element when predators encounter the moths? First, signal visibility is likely to depend on prey behavior and posture. In general, moth hindwings are normally hidden during rest and the warning signals in hindwings are visible only when moths open their wings (Kang et al. 2017). However, *A. nigriceps* has orange wing spots on both their fore- and hindwings, so even if hindwings are hidden when the moths are resting, the warning signals in the forewings remain clearly visible (Figure 1). The visibility of the orange abdominal stripes during resting behavior is less straightforward: the stripes can be completely or partly covered by the forewings, or completely visible (L Hämäläinen pers. observation, Figure 1), but how common each of these resting postures is remains unknown. Similarly, we know little about how predators perceive the different warning signal elements when the moths are flying. In some cases, color patterns appear to blur when prey move with sufficient speed (flicker fusion effect; Titcomb et al. 2014; Umeton et al. 2019). The abdominal stripes of *A. nigriceps* could create this effect during flight, and in this case the overall color ratio of black and orange might be more important than the striped patterns. However, the moths appear to have a slow flight pattern (L Hämäläinen pers.

405 observation), although their escape flight speed and the visibility of abdomen during
406 flight sequence has not been quantified. Future work should therefore aim to determine
407 the visibility of each warning signal element during rest and flight as this is essential for
408 understanding their importance in predator decision-making.

409 How predators perceive different warning signal elements also depends on the
410 viewing context, such as distance to the prey and visual environment (Ruxton et al. 2018).
411 Many color patterns are visible only when predators are in close proximity to the prey
412 (Barnett et al. 2018a; Barnett et al. 2018b). For example, orange and black stripes of
413 aposematic cinnabar moth (*Tyria jacobaeae*) caterpillars are salient at close range, but
414 the patterns blend into the background when viewed from a distance (Barnett et al.
415 2018a). Similarly, the different elements of *A. nigriceps*' warning signal might be salient
416 only when predators are very close to the moths, and these distance-dependent effects
417 require further investigation. Another important factor that may influence warning signal
418 detectability and predator responses is the light environment (Rojas et al. 2014). For
419 example, birds choose to attack different color morphs of an aposematic wood tiger moth
420 (*Arctia plantaginis*) depending on the light conditions (Nokelainen et al. 2022).
421 Heterogeneity in the light environment and background might therefore influence the
422 salience of the different warning signals elements, and their importance for predators
423 could be context dependent. Finally, our experiment included only visual warning
424 signals. However, in many aposematic species these are combined with other signal
425 modalities, such as odours or chemical secretions (Rowe & Haplin 2013), which can have
426 interactive effects that change predator responses to visual signals (Rojas et al. 2019).
427 This might be the case also in *A. nigriceps* that secrete defensive neck fluids when
428 attacked (Binns et al. 2022). These secretions could include odour cues, and further
429 research is needed to understand the potential interactions between different signal

430 modalities, and whether this changes predator responses to different visual elements of *A.*
431 *nigriceps* warning signals.

432 Our study demonstrates that different warning signal elements may vary in their
433 salience to predators, and understanding selection pressures for prey warning coloration
434 requires investigating the function of each individual element (Winters et al. 2017). We
435 show that the orange abdominal stripes of *A. nigriceps* are an important warning signal
436 for noisy miners, which could lead to relaxed selection on orange wing spots and provide
437 one explanation for the variation in the wing spot size (Binns et al. 2022). However,
438 predators used the wing signal in their foraging decisions when the stripes were not
439 visible, and future work should aim to quantify the visibility of each warning signal
440 element in different contexts to understand their role in predator attack decisions. For
441 example, it is possible that the orange wing spots have protective value when the moths
442 are resting and their abdomen is not visible, whereas the abdominal stripes could be a
443 more salient cue during flight. Both color patterns could also have other functions than
444 warning coloration. Wing spots, for instance, could function as disruptive coloration,
445 which might provide another explanation for variable wing patterns, and the role of wing
446 spots and abdominal stripes of *A. nigriceps* in contexts other than antipredator defences,
447 such as in sexual selection, remains uninvestigated. Overall, our study suggests that
448 examining the individual roles of warning signal components may change our predictions
449 of the evolution of prey warning coloration and help understand the observed diversity of
450 warning signals in nature.

451

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467

468 ***Data availability***

469 Analyses reported in this article can be reproduced using the data provided by Hämäläinen
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471

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