RESEARCH ARTICLE

Functional Ecology | BRITISH ECOLOGICAL SOCIETY

An aposematic colour-polymorphic moth seen through the eyes of conspecifics and predators – Sensitivity and colour discrimination in a tiger moth

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Abstract

1. Although predation is commonly thought to exert the strongest selective pressure on coloration in aposematic species, sexual selection may also influence coloration. Specifically, polymorphism in aposematic species cannot be explained by natural selection alone.

2. Males of the aposematic wood tiger moth (*Arctia plantaginis*) are polymorphic for hindwing coloration throughout most of their range. In Scandinavia, they display either white or yellow hindwings. Female hindwing coloration varies continuously from bright orange to red. Redder females and yellow males suffer least from bird predation.

3. White males often have higher mating success than yellow males. Therefore, we ask whether females can discriminate the two male morphs by colour. Males approach females by following pheromone plumes from a distance, but search visually at short range. This raises the questions whether males discriminate female coloration and, in turn, whether female coloration is also sexually selected.

4. Using electroretinograms, we found significantly larger retinal responses in male than female *A. plantaginis*, but similar spectral sensitivities in both sexes, with peaks in the UV (349 nm), blue (457 nm) and green (521 nm) wavelength range.

5. According to colour vision models, conspecifics can discriminate white and yellow males as separate morphs, but not orange and red females. For moths and birds (*Cyanistes caeruleus*), white males are more conspicuous against green and brown backgrounds, mostly due to UV reflectivity, and red females are slightly more conspicuous than orange females.

6. The costly red coloration among females is likely selected by predator pressure, not by conspecifics, whereas male colour polymorphism is probably maintained, at least partly, by the opposing forces of predation pressure favouring yellow males, and female preference for white males. Whether or not the preference for white males is based on visual cues requires further testing.

7. The evolution of polymorphic aposematic animals can be better understood when the visual system of the species and their predators is taken into consideration.

KEYWORDS
Arctiid moths, colour polymorphism, colour vision, natural selection, predator pressure, sexual selection, spectral sensitivity

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1 | INTRODUCTION

Predation is commonly advocated as the strongest selective pressure on the evolution of conspicuous coloration in aposematic organisms (Poulton, 1887; Ruxton, Sherratt, & Speed, 2004). However, in aposematic species with variable coloration within a given population (colour polymorphism), this principle is challenged by the difficulty for predators to learn several colour morphs (Endler & Mappes, 2004; Lindström, Alatalo, Lyytinen, & Mappes, 2001; Mallet & Joron, 1999). Previous studies have pointed at sexual selection as an alternative or additional selective pressure influencing the maintenance of colour variation (Crothers & Cummings, 2013; Jiggins, Naisbit, Coe, & Mallet, 2001; O'Donald & Majerus, 1984). This could be particularly relevant for species displaying sexual dichromatism, colour polymorphism in one of the sexes or both (Maan & Cummings, 2009; Nokelainen, Hegna, Reudler, Lindstedt, & Mappes, 2012; Rojas & Endler, 2013), as natural selection may favour one colour morph while sexual selection favours another (Crothers & Cummings, 2013; Nokelainen et al., 2012). Moreover, each of the different morphs might exploit receiver biases or limitations in the receivers' perceptual systems, especially while searching for mates (Limeri & Morehouse, 2014).

The wood tiger moth *Arctia plantaginis* (formerly *Parasemia plantaginis*; Rönkä, Mappes, Kaila, & Wahlberg, 2016) is an aposematic, colour-polymorphic and sexually dimorphic arctiid moth with a wide distribution across the Holarctic. Adults of *A. plantaginis* are active and mate from mid-June until the end of July in most of their range. They are diurnal-crepuscular, meaning that females release pheromones towards the evening hours, whereas males already start flying during daytime (Conner, 2009). Male flight activity is highest between 18 and 22 hr, and female calling and mating activity peak at 20 hr (own observations from the field, Gordon, Kokko, Rojas, Nokelainen, & Mappes, 2015; Rojas, Gordon, & Mappes, 2015). At this time of the year, nights in central and northern Europe are short; at the latitude of Helsinki (60°), the sun does not set before 22 hr, and nocturnal light levels are never reached.

Adult wood tiger moths have a conspicuous black-and-white pattern on the forewings and red, orange, yellow, white or black hindwings. In some populations, males are monomorphic, but in large parts of Europe, two male colour morphs with either white or yellow hindwings co-occur (Hegna, Galarza, & Mappes, 2015). In Finnish populations, frequencies of yellow and white morphs vary from 60:40 to 25:75 (Nokelainen, 2013). The hindwing colour of females in Europe ranges continuously from orange to red within populations, except for Scotland, where all females are yellow, and Georgia, where all females are red (Lindstedt et al., 2011; Hegna et al., 2015; B. Rojas, personal observation).

Both adult males (Nokelainen et al., 2012; Rojas et al., 2017) and females (Brain, 2016; Lindstedt, Reudler Talsma, Ihalainen, Lindström, & Mappes, 2010) are unpalatable to bird predators, which learn the moths' hindwing coloration as a warning signal (Lindstedt et al., 2011; Nokelainen, Valkonen, Lindstedt, & Mappes, 2014; Nokelainen et al., 2012; Rönkä, De Pasqual, Mappes, Gordon, & Rojas, 2018). Although the coloration of *A. plantaginis* has mostly been studied within the context of aposematism and predator–prey interactions (i.e. natural selection), considering birds as the main signal receivers (e.g. Nokelainen et al., 2012, 2014), wing coloration may also have a function in intraspecific communication (e.g. sexual selection). Experiments suggest that white males have a higher mating success than yellow males (Gordon et al., 2015; Nokelainen et al., 2012), particularly if males experience stress. However, these experiments did not reveal whether females based their choice on colour or on other properties related to the colour morph.

While females attract males from afar by pheromones, approaching males visually search for females at short range in the vegetation (B. Rojas & J. Mappes, personal observation). Males might be choosy about which female(s) to approach, and females may or may not mate with a male they have attracted (Gordon et al., 2015). Altogether, this raises the question whether wing coloration plays a role in mate detection and choice. Addressing this question requires that we know how tiger moths perceive their own wing colours.

In this study, we investigated the eyes of *A. plantaginis* using histology and electroretinograms (ERGs). We determined the spectral sensitivity of the retina, measured the wing reflectance and used a colour vision model to estimate how well the moths can detect conspecifics against natural backgrounds, and discriminate between their colour morphs. We also assessed how well a natural predator, the blue tit *Cyanistes caeruleus*, can detect the different colour morphs of the wood tiger moth. Our results allow predictions on the opposing selective forces that act on the colour morphs and maintain colour polymorphism within populations.

2 | MATERIALS AND METHODS

2.1 | Animals

We investigated animals from a stock of wood tiger moths from southern and central Finland that has been kept at the University of Jyväskylä, Finland, since 2013, with wild individuals being added every year during the field season and crosses between brothers and sisters being systematically avoided. Frequencies of yellow and white morphs were kept to approximately 50:50. Larvae were raised under greenhouse conditions and fed dandelion (*Taraxacum* sp.) leaves *ad libitum* (for more details, see Lindstedt et al., 2010). Rearing containers were checked and cleaned daily until the larvae pupated. Pupae were kept at 24°C in transparent plastic boxes lined with paper towels. After the moths had eclosed, straightened their wings and hardened, they were subjected to experiments the same day or transferred to a dark chamber and stored at 8°C for later investigation. Moths used for electrophysiology originated from the 2nd, moths for anatomy from the 9th and those for reflectance measurements from the 11th generation of the laboratory stock.

2.2 | Eye histology

Three male and three female *A. plantaginis* were decapitated, and their heads dissected and fixated in 2% glutaraldehyde, 2%

formaldehyde and 2% sucrose in 0.15 M sodium cacodylate buffer for 12–24 hr. After repeated rinsing in buffer, the heads were embedded in epoxy resin. Vertical sections of 3 μm thickness were taken with a Reichert Ultracut microtome using glass knives. The sections were placed on a slide, dried on a hot plate, stained with toluidine blue and photographed under a light microscope. Rhabdom lengths were measured from micrographs using Image J150 (NIH, Bethesda, MD, USA), following the course of the rhabdom from the base of the crystalline cone to the basement membrane.

2.3 | Electroretinogram (ERG) recordings

For electrophysiology, a male (n = 8) or female (n = 6) adult moth was mounted on a holder and placed in a light-tight, dark Faraday cage. To explore regional differences in the retina, we inserted a tungsten recording electrode either in the ventral (n = 9) or in the dorsal (n = 5) half of one compound eye. The eye was illuminated via the central fibre of a light guide, whose angular position and distance were adjusted to maximize responses to flashes of 40 ms duration separated by pauses of 5 s. After dark adaptation for 30 min, the spectral sensitivity was measured up to four times (see Supporting Information Methods) presenting narrowband spectral flashes of equal photon flux as described previously (Jakobsson, Henze, Svensson, Lind, & Anderbrant, 2017; Telles et al., 2014). Preceding and following each spectral series, we determined a voltage response–intensity (V-log I) relationship using flashes of white light with increasing intensity I. Response characteristics of the dark-adapted retina were investigated 3–20 times for each individual, with 10 or more datasets for most animals recorded at different times of the day.

To isolate the contribution of short-wavelength receptors to the ERG, we repeated the recording protocol with continuous adaptation light from one of four light emitting diodes (LEDs) presented through the peripheral fibres of the light guide in the order red, amber, green and blue (see Supporting Information Methods). Each LED was switched on 5 min before a series of recordings started and operated to produce increasing intensities at the position of the eye in subsequent experiments. In between sets of experiments and in the end, we monitored the state of the animal by repeatedly recording spectral control series from the dark-adapted retina.

2.4 | ERG analyses

ERGs were analysed by custom-made Matlab scripts (R2013b or R2015b, The MathWorks, 160 Natick, MA, USA). The response amplitude V was calculated as the absolute value of the potential change from the baseline at stimulus onset to maximal hyperpolarization. To assess the stability of the recordings and to document the dynamic range and saturation level of responses, a Naka-Rushton function was fitted to each V-log I dataset based on a nonlinear least-squares solution.

For the dark-adapted retina, we calculated the asymptotic maximal response V_{max}, the intensity K that elicited a half-maximal response, and the slope of the Naka-Rushton function at K. We tested

the effect of *sex* and *measurement number* (repetitions) on these three parameters using linear mixed-effects models with a nested design (*measurement number* nested within *individual*). To test for time dependence, we fitted linear regression models to the data, with the effect of *sex* adjusted for the *time of day* in hours after midnight. All statistical models were implemented in R v3.3.3. (R Core Team 2017).

Based on the Naka-Rushton function fitted to the V-log I datasets obtained before and after each spectral series, we converted response amplitudes for spectral flashes into normalized sensitivities as described in Telles et al. (2014). Templates (Govardovskii, Fyhrquist, Reuter, Kuzmin, & Donner, 2000) were used to estimate the sensitivity maxima (λ_α) of receptor types contributing to the averaged sensitivity curves (n = 4) by a nonlinear least-squares approach (see Supporting Information Methods). To get the best estimate for λ_α of a specific receptor type, we selected curves with minimal contributions from other receptors.

2.5 | Reflectance measurements

Spectral reflectance $s(\lambda)$ of 16 white and 10 yellow males, as well as 8 females classified as orange and 10 females classified as red, was measured in the range of 300 to 700 nm, with 1 nm resolution, using an Ocean Optics USB4000 spectrometer (Dunedin, FL, USA) connected to a PX-2-pulsed Xenon lamp (for further details see Lindstedt et al., 2011; Nokelainen et al., 2012). A Spectralon TM white reflectance standard (Labsphere, Congleton, UK) was used for calibration.

2.6 | Model calculations

The quantum catch Q_i of photoreceptor i (i = UV, blue, green receptor) is given by:

$$Q_i = k_i \int_{300}^{700} R_i(\lambda)s(\lambda)I(\lambda)d\lambda, \tag{1}$$

where λ is wavelength, R is receptor sensitivity, s is the reflectance spectrum of the specimen, I is the illumination spectrum, and k is a scaling factor given by adaptation to the background spectrum s_b:

$$k_i = \frac{1}{\int_{300}^{700} R_i(\lambda)s_b(\lambda)I(\lambda)d\lambda}. \tag{2}$$

We estimated visual contrast using a receptor noise-limited (RNL) model of colour discrimination (Vorobyev & Osorio, 1998). In this model, we suppose that colour discrimination limits are set by receptor noise that propagates into higher neuronal levels via retinal opponent mechanisms. We assume a loglinear relationship between receptor quantum catch and receptor signals, $f = \ln(Q_i)$, so that the contrast Δf between two stimuli s_1 and s_2 is

$$\Delta f = \ln\left(\frac{Q_i s_1}{Q_i s_2}\right). \tag{3}$$

Each receptor mechanism is limited by a Weber fraction ω given by receptor noise:

$$\omega_i = \frac{e_i}{\sqrt{\eta_i}}, \tag{4}$$

where e is the noise in each receptor type and η is the proportion of each receptor channel in the retina. As receptor noise and spatial summation are unknown for *A. plantaginis*, we set noise in the green receptor channel to 0.1, the level measured in honeybees (Vorobyev, Brandt, Peitsch, Laughlin, & Menzel, 2001). Assuming that each ommatidium has one UV, one blue and seven green receptors, as in various other moths (Briscoe, 2008; Jakobsson et al., 2017; Warrant, Kelber, & Kristensen, 2003; but see Belušiç, Šporar, & Meglič, 2017), we set the receptor proportions to 1:1:7 (UV:blue:green receptors). Given the uncertainty of noise in moth photoreceptors, we focus on relative chromatic contrast and also test whether our conclusions hold even for a flat receptor abundance ratio of 1:1:1.

Colour contrast S is given by:

$$S^2 = \frac{\omega_{UV}^2 \left(\Delta f_{green} - \Delta f_{blue}\right)^2 + \omega_{blue}^2 \left(\Delta f_{green} - \Delta f_{UV}\right)^2 + \omega_{green}^2 \left(\Delta f_{blue} - \Delta f_{UV}\right)^2}{\left(\omega_{UV}\omega_{blue}\right)^2 + \left(\omega_{UV}\omega_{green}\right)^2 + \left(\omega_{blue}\omega_{green}\right)^2}. \tag{5}$$

The unit for colour contrast is jnds (just noticeable differences), and 1 jnd is defined as the colour contrast at detection threshold. For estimating the contrast in the visual system of the blue tit (*Cyanistes caeruleus*), we used the model for tetrachromatic bird vision (given in Supporting Information Methods).

We calculated colour contrast under standard daylight illumination (d65; Wyszecki & Stiles, 2000) using two background spectra corresponding to green and brown vegetation. Our conclusions do not change when assuming the illumination in a deciduous forest (Håstad, Victorsson, & Ödeen, 2005), daylight d75, or light spectra typical for sunset or twilight (Johnsen et al., 2006) instead (Figure S3).

We determine the position of colour loci (position of colours in the colour space) in a chromaticity diagram according to the following axes:

$$X_1 = A\left(f_{green} - f_{blue}\right)$$

$$X_2 = B\left(f_{UV} - \left(af_{green} + bf_{blue}\right)\right), \tag{6}$$

where the coefficients (A, B, a, b) scale the chromaticity diagram according to the receptor noise-limited model such that the Euclidean distance of 1 between two colour loci corresponds to 1 jnd (Equation 5):

$$A = \sqrt{\frac{1}{\left(\omega_{blue}\right)^2 + \left(\omega_{green}\right)^2}}, \tag{7}$$

$$B = \sqrt{\frac{\left(\omega_{blue}\right)^2 + \left(\omega_{green}\right)^2}{\left(\omega_{UV}\omega_{blue}\right)^2 + \left(\omega_{UV}\omega_{green}\right)^2 + \left(\omega_{blue}\omega_{green}\right)^2}}, \tag{8}$$

$$a = \frac{\left(\omega_{blue}\right)^2}{\left(\omega_{blue}\right)^2 + \left(\omega_{green}\right)^2}, \tag{9}$$

$$b = \frac{\left(\omega_{green}\right)^2}{\left(\omega_{blue}\right)^2 + \left(\omega_{green}\right)^2}. \tag{10}$$

Achromatic contrast was calculated as Michelson contrast (see Supporting Information Methods).

3 | RESULTS

3.1 | Eye anatomy

We inspected vertical sections of the compound eyes and optic lobes of male and female *Arctia plantaginis* under the light microscope. The light-adapted eyes (Figure 1) are close to spherical and have a cornea of ≈18 μm thickness, a crystalline cone of about twice this length, and 76±8 μm long rhabdoms in females (*n* = 10), but 100±11 μm long rhabdoms in males (*n* = 10). As no clear zone could be detected, the eyes likely function as apposition eyes. The sections of the heads also revealed that the second optic neuropil, the lamina, is spatially not directly adjacent to the retina in *A. plantaginis* (Figure 1).

3.2 | Electroretinograms (ERGs)

ERGs from the compound eyes of *A. plantaginis* consisted of monophasic hyperpolarizations (Figure 2a), indicating that we recorded
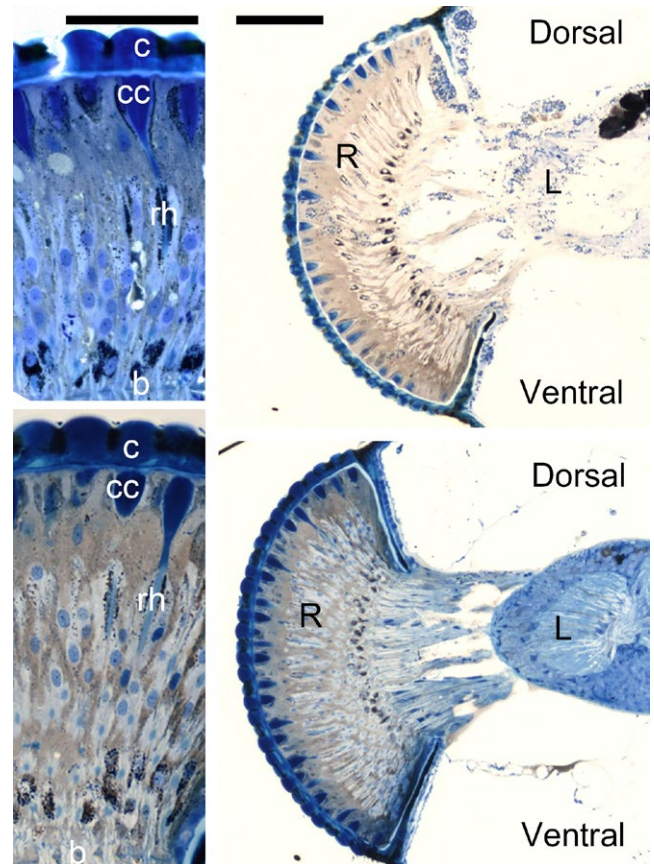
FIGURE 1 Vertical sections of the compound eyes of female (top) and male (bottom) *Arctia plantaginis*. Left, enlarged region of the eye with c cornea; cc, crystalline cone; rh, rhabdom; b, basement membrane; scale bar 50 μm. Right, eyes (R retina) and first optic neuropil, lamina (L), scale bar 100 μm
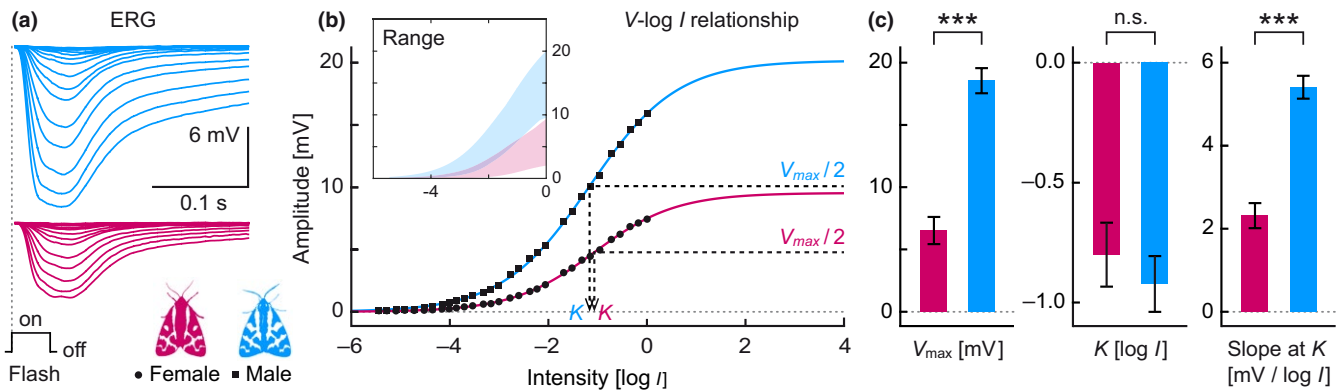
FIGURE 2 Sex differences in response strength of the dark-adapted retina in *Arctia plantaginis*. (a) Electroretinograms (ERGs) from the compound eyes of a male and a female moth in response to 40 ms flashes of white light with increasing intensity. For clarity, only every second ERG is shown. (b) Amplitudes V (potential changes from the baseline at stimulus onset to maximal hyperpolarization) plotted against intensity I for the datasets partially shown in (a). We fitted a Naka-Rushton function to each dataset and estimated the asymptotic maximal response V_{max}, the intensity K that elicited a half-maximal response, and the *Slope* of the curve at K. The inset illustrates the range of data from all eight males and six females. (c) Sex differences of the parameters V_{max}, K and *Slope* at K (mean ± standard error) tested by linear mixed-effects models

TABLE 1 Influence of sex and measurement number on response properties of the dark-adapted retina in the compound eyes of *A. plantaginis* tested by linear mixed-effects models. For a definition of V_{max}, K and *Slope* at K, see Figure 2

	Mean difference	Standard error	95% confidence interval	t	df	p value
V_{max} [mV]						
Sex	12.0	1.4	9.1, 15.0	8.87	12	<.001
Measurement no.	0.1	0.1	−0.2, 0.3	0.54	130	.59
K [log I]						
Sex	−0.1	0.2	−0.5, 0.3	−0.72	12	.48
Measurement no.	−0.02	0.01	−0.1, −0.001	−2.10	130	.04
Slope at K [mV/log I]						
Sex	3.1	0.4	2.2, 4.0	7.69	12	<.001
Measurement no.	0.1	0.04	−0.03, 0.1	1.23	130	.22

photoreceptor responses only. The rather long distance between retina and lamina (see Figure 1) is probably the reason why our electrode did not pick up signals of downstream neurons.

We observed striking differences in response strength between males ($n = 8$) and females ($n = 6$; Figure 2; data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.s46t627). In the dark-adapted retina, the estimated maximal response V_{max} to flashes of white light was more than twice as large in males (mean ± standard error: 18.6 ± 1.0 mV) as in females (6.5 ± 1.1 mV; values are based on a linear mixed-effects model accounting for repeated measurements per *individual*). This difference of 12 mV was highly significant ($t_{(12)} = 8.86$, $p < .001$, Table 1). K, the intensity that elicits a half-maximal response ($V_{max}/2$) and marks the turning point of the Naka-Rushton function fitted to the response–intensity (V-log I) relationship, did not differ significantly between the sexes. However, the slope of the curve at K was significantly higher for males than females (mean difference 3.1 mV/log I, $t_{(12)} = 7.69$, $p < .001$, Table 1 and Figure 2). Regardless of the statistical model, *sex* had a robust

effect on V_{max} and on the *Slope* at K, but not on K itself (compare Table 1 and Table S1). This was independent of the number of measurements or the time of day, at least for the 16 hrs between 10 and 2 hr (the next day), for which we have recordings. *Measurement number* had a significant effect on K, but the p-value was high and the size of the effect minimal (mean difference −0.02 mV/log I, $t_{(130)} = -2.10$, $p = 0.04$, Table 1).

The spectral sensitivity curves obtained under dark adaptation and adaptation to different intensities of red, yellow, green or blue light were all in agreement with the assumption of three photoreceptor types expressing visual pigments with peak sensitivities in the UV, blue and green range (e.g. see Figure 3; data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.s46t627). Adding another spectral receptor type to the models did not considerably improve the fit. Differences in the wavelengths of maximal sensitivity λ_α between males and females, and dorsal and ventral eye regions were within the limits of experimental accuracy. We therefore averaged all results for each receptor type leading to a λ_α-value
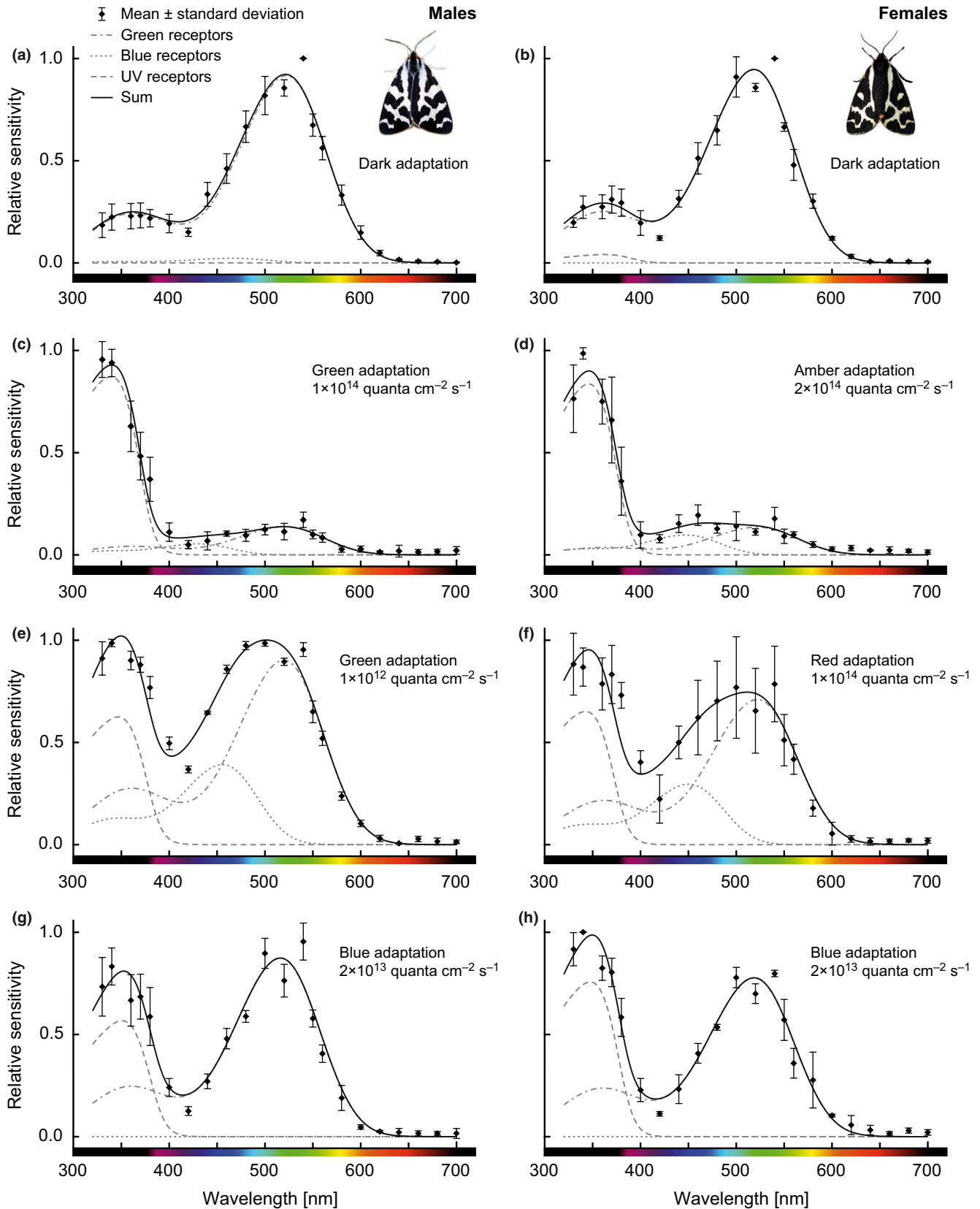
FIGURE 3 Spectral sensitivities of male (left) and female (right) *Arctia plantaginis* derived from ERGs. For each individual, averaged measurements (*n* = 4) from one compound eye under different adaptation states were selected to estimate the wavelength of maximal sensitivity (λ_α) for three spectral types of photoreceptor. Example curves used to assess λ_α of the green receptors (a, b), λ_α of the UV receptors with λ_α of the green receptors predetermined (c, d), and λ_α of the blue receptors with λ_α of the green and UV receptors predetermined (e, f). (g, h) Examples, in which the blue receptors were suppressed by blue light, isolating the responses of the UV and green receptors
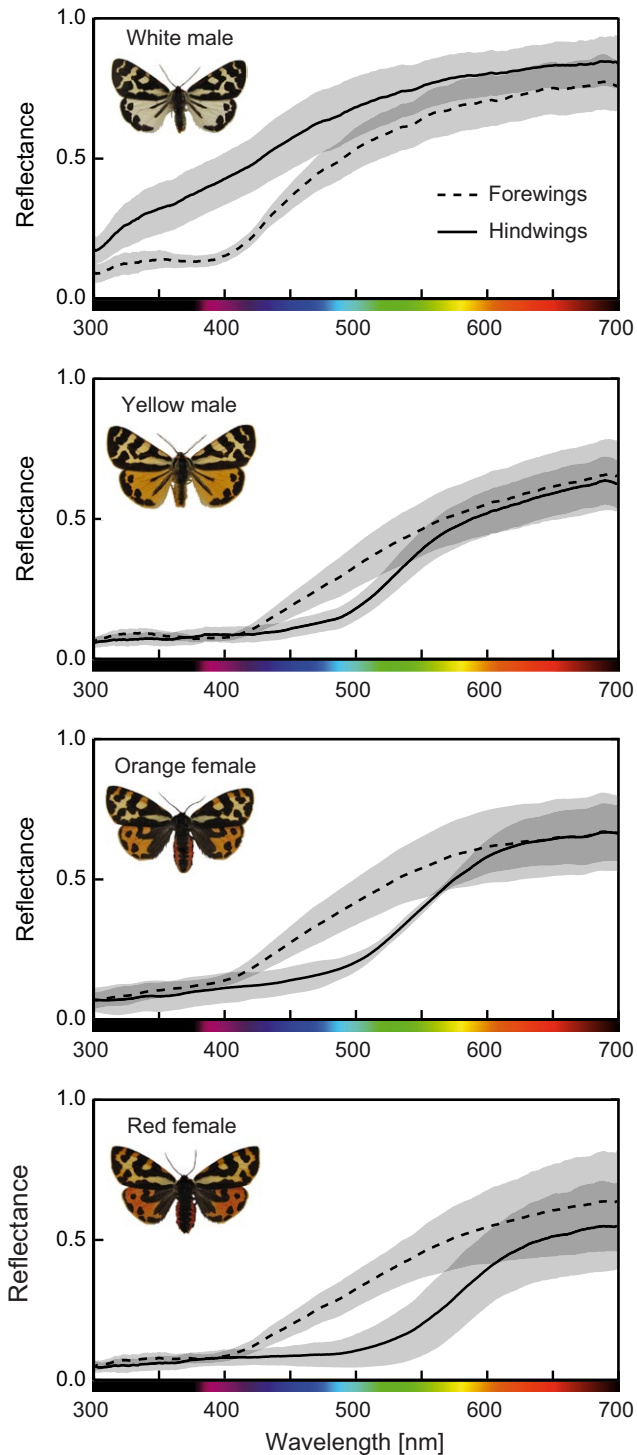
FIGURE 4 Reflectance of forewing (dashed lines) and hindwing (solid lines) colours of male and female *Arctia plantaginis*. Lines give averages and grey areas standard deviations. Data from 16 white males, 10 yellow males, 8 orange females and 10 red females

of 521 ± 2 nm (mean ± standard deviation, n = 14) for the green receptor, 456 ± 7 nm (n = 14) for the blue receptor and 349 ± 2 nm (n = 13) for the UV receptor. Note that the peak sensitivities (λ_α) of the three receptors are not equally spaced across the spectrum. Instead, the sensitivity peak of the blue receptor is shifted to longer

wavelengths (i.e. red-shifted) by 21 nm. For the dark-adapted eye, the sensitivity peak of the green receptor type was 11 to 12 times as high as the sensitivity peak of the UV and the blue receptor type, indicating a larger number of green receptors, likely also with bigger rhabdoms.

3.3 | Wing coloration

As in previous studies on the species, we classified males after inspection by the human eye as belonging to the white or the yellow morph, and females as either orange or red. While we measured very similar reflectance curves from the forewings of all morphs, hindwings differed. In particular, the hindwings of white males reflected more UV than those of any other morph (Figure 4; data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad. s46t627).

We used the receptor sensitivities determined by ERGs (Figure 5a) to estimate the colour contrast of white and coloured wing patches of *A. plantaginis* in daylight illumination (Figure 5c) with the RNL model of colour discrimination (Vorobyev & Osorio, 1998). This model provides a quantitative measure of chromatic contrast while achromatic (or luminance) contrast is ignored. Because one of the input values, the noise level in each photoreceptor channel, is unknown for *A. plantaginis*, we can only give a rough estimate of absolute discrimination thresholds. Instead, we focus on relative colour contrast, which is unaffected by this uncertainty (Table S2, Figure S2). We do not predict colour contrasts for black wing regions, assuming that the contrasts between black and either white or coloured areas are mostly detected by the achromatic channel.

We first address the discrimination between wing colours of different morphs, and plot all colours in the colour space described by the RNL model. We assume that the moths can discriminate distinct morphs by colour, if the average contrast between the colours of different morphs exceeds the contrast between colours within each morph such that morph colours form non-overlapping clusters.

This is not the case for the forewing colours (Table 2, Figure 6a). Hindwing coloration is more variable (Fig 6b) differing most strongly in the stimulation of the UV receptor, which is evident from the oblique-vertical distribution of colour loci in colour space (Figure 6b). The average contrast between hindwing colours of white and yellow males is higher than the contrast within each of these morphs (Table 2), and—with the exception of two outliers—the hindwing colour of the white morph forms a distinct cluster in colour space, separated from all other morphs (Figure 6b). Thus, we assume that *A. plantaginis* likely discriminates white and yellow males as two different colour morphs, just as we do, based on human vision. The orange and red hindwing colours of females, in contrast, do not form distinct clusters, but largely overlap (Table 2, Fig 6b) and build a continuum. While conspecifics will be able to discriminate certain orange and red individuals, they will not be able to categorize them visually as belonging to two morphs.
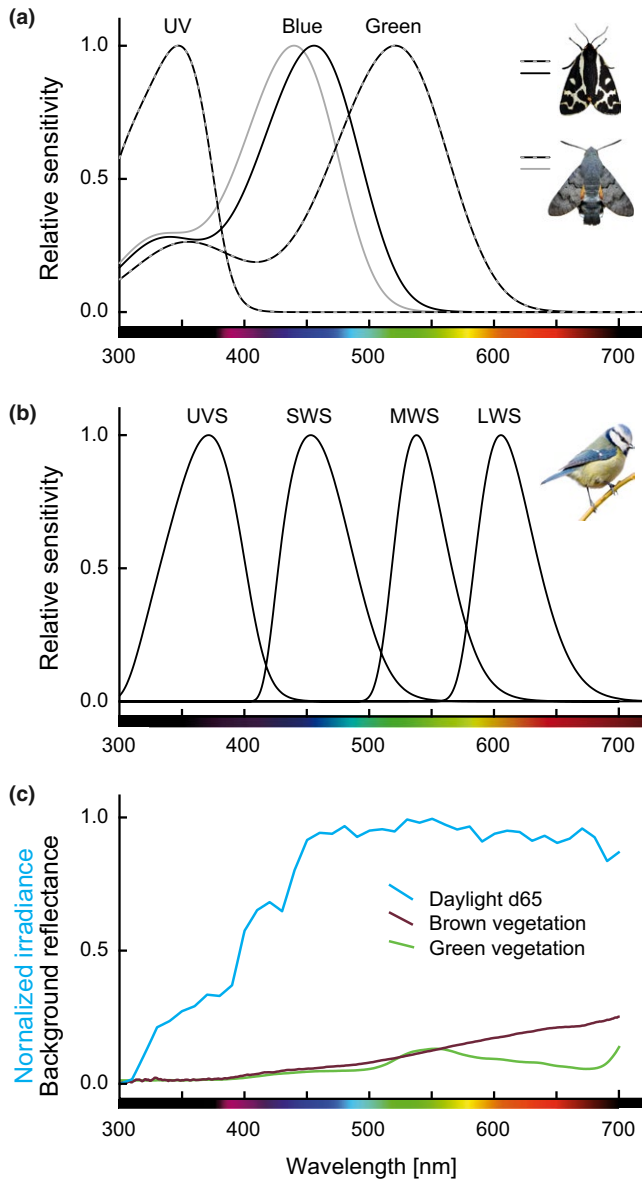
FIGURE 5 Data used in model calculations. (a) Spectral sensitivities of photoreceptors in the compound eyes of the wood tiger moth (*Arctia plantaginis*) and the hummingbird hawkmoth (*Macroglossum stellatarum*; data from Telles et al., 2014). The sensitivities of UV and green receptors based on the template (Govardovskii et al., 2000) are the same in both species, but the sensitivity of the blue receptors in *A. plantaginis* is red-shifted. (b) Spectral sensitivities of the four single cones in the blue tit (*Cyanistes caeruleus*; data from Hart et al. 2000). UVS, ultraviolet sensitive; SWS, short-wavelength sensitive; MWS, middle-wavelength sensitive; LWS, long-wavelength sensitive. (c) Reflectance spectra of green and brown vegetation and irradiance of daylight (d65; Wyszecki & Stiles, 2000)

Next, we investigated the colour contrasts of the wings against different natural backgrounds (Figure 5c). For the moths themselves, wing colours have very similar chromatic contrasts against green and brown backgrounds, and hindwings are more conspicuous than fore-wings (red symbols in Figure 7). All achromatic contrasts are higher than 0.1 and thus easily discriminable (Figure S1).

To understand whether the red-shift of the blue receptor of *A. plantaginis* enhances its ability to detect and differentiate conspecifics, we compared colour discrimination of *A. plantaginis* with that of *Macroglossum stellatarum*, a moth with equally spaced receptor sensitivities (Figure 5a, data from Telles et al., 2014) (white symbols in Figure 7). Assuming the same noise levels, we found no significant differences between these two visual systems, neither in the discrimination of wing colours against green or brown backgrounds (Figure 7), nor in the discrimination between wing patches (Table S3). All results hold true even if we assume different relative numbers of receptor types (Figure S2) or different illumination spectra (Figure S3).

Finally, we asked whether the blue tit, a predator with tetrachromatic colour vision (for receptor sensitivities, see Figure 5b), can detect specific morphs better than others. For this bird, colour contrast of all except the white hindwings is higher against the green than the brown background, and all hindwing colours are more conspicuous than forewing colours on both backgrounds (blue symbols in Figure 7). Achromatic contrasts are also high (Figure S1), but birds are generally thought to use chromatic rather than achromatic contrast.

4 | DISCUSSION

Classical approaches have invoked predation as the most representative selective pressure favouring aposematic coloration. However, this approach falls short to explain the intrapopulation variation in the coloration of some aposematic species, as this variation is expected to hinder predator learning and the subsequent avoidance of aposematic prey. Sexual selection has been suggested as a pressure capable of counterbalancing predation, thus allowing multiple morphs of an aposematic species to coexist. This could be the case in the polymorphic wood tiger moth, where one morph appears to be favoured by predators while the other morph seems to be preferred by females. In the present study, we investigated whether the moths can visually discriminate the colour morphs of conspecifics, a precondition for sexual selection to play a role in the maintenance of this polymorphism. Our results indicate that male and female wood tiger moths have similar spectral sensitivities and, while both sexes can discriminate the two male colour morphs, they are unable to distinguish orange from red female morphs. Furthermore, in the eyes of both conspecifics and predators, white males are more conspicuous against green and brown backgrounds, whereas red females are slightly more conspicuous than orange females. We discuss the implications of our findings below.

4.1 | Eye anatomy and sensitivity

Wood tiger moths appear to have functional apposition compound eyes (Figure 1), as the rhabdoms of the photoreceptors are in direct contact with the crystalline cones, without the clear zone that is typical for superposition eyes (Land & Nilsson, 2002). As superposition eyes are the general rule for moths (Warrant et al., 2003) including

TABLE 2 Colour contrast (jnds) between patches of forewings and hindwings in male and female wood tiger moths. For each comparison, we give the median colour contrast with the 25th and 75th percentiles in brackets

Male forewing colour			Female forewing colour		
	White	**Yellow**		**Orange**	**Red**
White	0.9 (0.3–1.6)	1.0 (0.7–1.4)	Orange	1.1 (0.5–1.8)	1.1 (0.6–1.4)
Yellow		0.7 (0.3–1.2)	Red		0.5 (0.2–0.9)
Male hindwing colour			**Female hindwing colour**		
	White	**Yellow**		**Orange**	**Red**
White	0.1 (0.1–0.3)	2.6 (1.8–3.3)	Orange	1.4 (0.6–2.7)	1.7 (1.1–2.5)
Yellow		0.9 (0.5–1.5)	Red		0.5 (0.2–0.8)
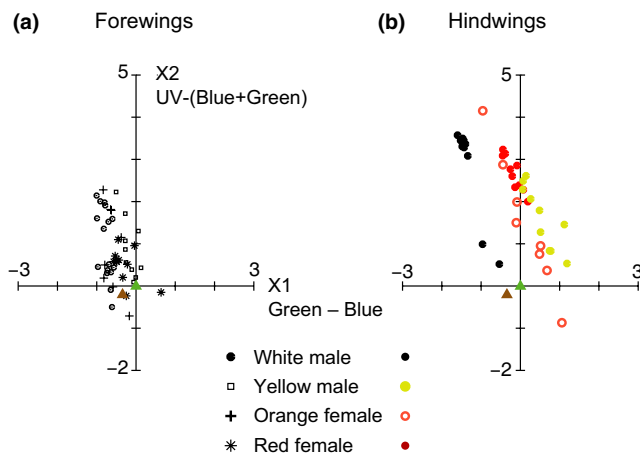
FIGURE 6 The loci of forewing (a) and hindwing (b) colours in the chromaticity diagram of *Arctia plantaginis*. Each data point represents the colour locus given by a reflectance spectrum illuminated by daylight d65. Receptors are adapted to a green background (green triangle); the colour locus of the brown background (brown triangle) is shown for comparison. The Euclidean distance between loci equals their contrast expressed in jnds (see Methods for details). Colour loci are visualized on specific opponent axes in the chromaticity diagram, but the contrast between colours is set by receptor noise and independent of specific colour opponency (Vorobyev & Osorio, 1998)

Arctiidae (D.-E. Nilsson, personal communication), this result comes as a surprise, although similar cases have been reported (Warrant et al., 2003). Males have slightly longer rhabdoms than females, which should make their photoreceptors ≈20% more sensitive. However, this can only partly explain why the signals recorded from the dark-adapted retina were more than 100% larger in males than females (Figure 2). Further studies are required to understand the physiological basis of the observed sex-specific differences.

Our analyses suggest that the eyes of both sexes are optimized for similar light intensities, because the linear working range of male and female eyes is centred on the same intensity K (Table 1, Figure 2). However, the slope of the curves is steeper in males than females, indicating that intensity differences translate into larger response differences, which might increase contrast sensitivity.

Higher (absolute and contrast) sensitivity of male eyes fits well to the natural behaviour of the moths. Females, which carry a heavy weight of developed eggs when they eclose, mostly sit on the vegetation and emit pheromones until they mate, lay eggs and die. Their flight activity is limited and not important for survival or reproduction. Males instead fly to find a mate and have to detect them visually in dense vegetation. For flight control and for detecting females, males need sensitive eyes.

Our results show sex differences in the response strength of the photoreceptors to flashes of white light but not in their spectral tuning (Figure 3). We have found evidence for three spectral types of photoreceptor, with sensitivity peaks for UV, blue and green light. More than three receptor types have been described in many butterflies (Arikawa & Stavenga, 2014), yet rarely in moths (Belušič et al., 2017; Jakobsson et al., 2017; Langer, Hamann, & Meinecke, 1979; Warrant et al., 2003). We cannot exclude that we missed additional receptor types in *A. plantaginis*, but these receptors, if present, either have a spectral sensitivity close to those characterized here or do not contribute substantially to the ERG.

While the spectral sensitivities of the UV and green receptors of *A. plantaginis* are similar to those found in sphingid moths (*Manduca sexta*: White, Xu, Munch, Bennett, & Grable, 2003; *Macroglossum stellatarum*: Telles et al., 2014), the sensitivity of the blue receptor is red-shifted (Figure 5a). It is normally assumed that equally spaced photoreceptor sensitivities allow for optimal discrimination of general colour stimuli (e.g. Barlow, 1982). However, our models comparing *A. plantaginis* with *M. stellatarum* revealed no significant differences in discriminability of colours and thus no obvious effect of the red-shift of the blue receptor in *A. plantaginis* (Figure 7).

4.2 | Wing coloration in the eyes of conspecifics

Seen with the eyes of *A. plantaginis*, forewings do not differ between morphs, but white and yellow males have clearly distinct hindwing coloration (Table 2, Figure 6). Mostly because of the stronger UV reflection of their hindwings, white males have a higher chromatic contrast than yellow males against green and brown backgrounds (Figure 7). Nokelainen et al. (2012) showed the opposite for a flat white background spectrum. Thus, yellow males have a higher
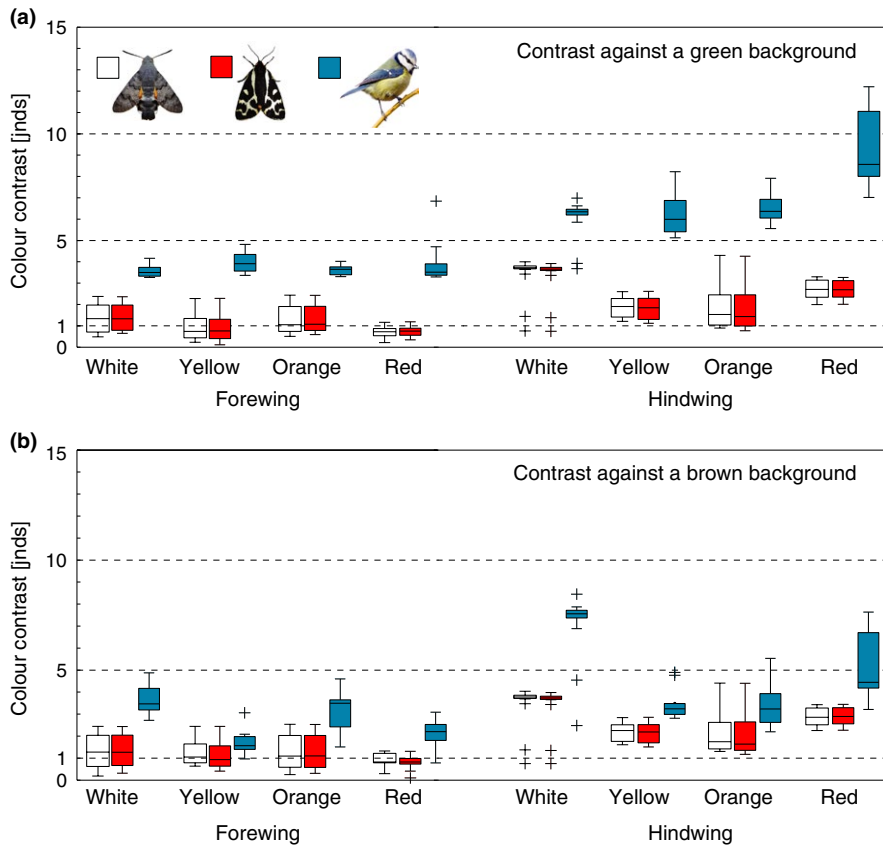
FIGURE 7 Colour contrast between wing patches and (a) a green, or (b) a brown background in *Macroglossum stellatarum* (white), *Arctia plantaginis* (red) and *Cyanistes caeruleus* (blue). Contrast was calculated for forewing and hindwing colours from 16 white and 10 yellow males, and 8 orange and 10 red females. Boxes show the 25th, 50th and 75th percentiles of colour contrast with whiskers spanning the range of the distributions to a maximum of ± 1.5 times the interquartile range (75th-25th). Data points outside this interval are counted as outliers (crosses). The absolute threshold of detection is set to 1 jnd; however, in the moths, this limit is uncertain as the receptor noise levels are not well known (see Methods)

chromatic contrast than white males on the white bark of a birch tree, but a lower contrast on green or brown vegetation. This could have implications for sexual selection in the species (Gordon et al., 2015; Nokelainen et al., 2012). Our results also show that orange and red females cannot be discriminated as distinct colour morphs (Table 2, Figure 6). Therefore, we suggest that female hindwing coloration is of little importance for mate selection.

It is interesting to note that the high contrast of the white hindwings against green and brown backgrounds and to the hindwing colours of other morphs mostly results from high UV reflection. Obviously, our anthropomorphic view and naming of colour morphs do not always conform with their appearance to conspecifics and can be misleading when searching for communication signals.

4.3 | Aposematic vs. intraspecific signals

To a bird, white and yellow males of *A. plantaginis* have a similar contrast against a green background (blue symbols in Figure 7). Blue tits hesitate much longer before attacking yellow than white males presented on a green background (Nokelainen et al., 2012), but the predation on both morphs depends on the local bird community (Nokelainen et al., 2014). We suggest that colour *per se*, and perhaps the black-and-yellow pattern, and *not* the contrast against the background determines the aposematic function of the hindwings. Alternatively, the recently observed strong pyrazine odour of yellow males, which is more effective than that of white males (Rojas et al., 2017), may explain the hesitation of birds when attacking yellow

moths. By contrast, the strong UV reflection of the hindwings of white males, though making them conspicuous to both females and birds, is unlikely to contribute to avoidance learning. UV is not an efficient warning signal on its own (Lyytinen, Alatalo, Lindström, & Mappes, 2001) and can even attract birds rather than stop them from attacking (Lyytinen, Lindström, & Mappes, 2004; Olofsson, Vallin, Jakobsson, & Wiklund, 2010). Nevertheless, our results support the role of natural selection in the evolution of hindwing colour in *A. plantaginis* males and suggest that sexual selection may play a role, too.

Our model calculations indicate that the variation in female hindwing coloration is perceived as a continuum by conspecifics; it is thus unlikely to contribute to mate choice. Predation, however, seems to represent a selection pressure on female coloration, as birds avoid red females more often than orange females (Lindstedt et al., 2011) and learn the red colour faster (Rönkä et al., 2018). While the red pigmentation warns birds about unprofitability (Lindstedt et al., 2011), it is costly and depends on larval diet, such that larvae reared on a diet high in iridoid glycosides become adults with paler (less red) wing pigmentation (Lindstedt et al., 2010).

Forewings, on the other hand, are highly conspicuous because of the high achromatic (luminance) contrast between white and black patches (Figure S1). Naïve birds are more strongly attracted to patterns combining yellow or red with black-and-white than with grey patches. However, they learn to avoid the former much faster (Zylinski & Osorio, 2013). The combination of black-and-white forewings with red or yellow hindwings may thus be an efficient learning

cue for birds. In fact, birds readily avoid the forewing pattern of *A. plantaginis* (Hegna & Mappes, 2014).

The interplay of natural and sexual selection in the evolution of visual signals manifests in different ways across taxa. Brightly coloured models of red postman butterflies (*Heliconius erato*) attracted more males *and* deterred predators more efficiently than achromatic models and models of nonlocal morphs (Finkbeiner, Briscoe, & Reed, 2014). This suggests that predator deterrence (aposematism) and mate choice work in the same direction, which may explain why only one morph occurs in the studied population of this *Heliconius* species (Finkbeiner et al., 2014). In contrast, in small cabbage white butterflies (*Pieris rapae*), females prefer males that are more contrasting against the background. These males are also most conspicuous to birds, and thus in greater risk of predation (Morehouse & Rutowski, 2010), a situation resembling the balance between natural and sexual selection in the well-known case of Trinidadian guppies (Endler, 1983).

Opposing sexual and natural selection alone cannot explain the maintenance of local polymorphism, as this would require exactly equal strength of both selective pressures. Thus, additional selective or genetic factors are usually involved. In *A. plantaginis*, where yellow males, on average, have an advantage against predators while white males tend to have a mating advantage, gene flow and variable selection by predators seem to offer an explanation for polymorphism (Galarza, Nokelainen, Ashrafi, Hegna, & Mappes, 2014; Nokelainen et al., 2014). Our results on *A. plantaginis* vision confirm that male selection for female coloration is unlikely, but a female may use chromatic cues for mate choice once a male has approached her, favouring the UV-reflecting white males. Whether females use visual cues in mate selection, or whether other traits explain mating success of white males better, requires further investigation.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTIONS

J.M., B.R., M.J.H. and A.K. planned the study. J.M. and B.R. provided animals, wing spectra and ecological knowledge on the study species, A.K. studied eye histology, M.J.H. measured and analysed ERGs, and O.L. calculated colour vision models. All authors discussed the results, contributed to writing text and preparing figures and approved the publication. No author has any conflict of interest.

DATA ACCESSIBILITY

All original data used in this study that are not presented in the main text or the supplements, can be found on the Dryad Digital Repository: https://doi.org/10.5061/dryad.s46t627 (Henze et al., 2018).

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