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Cite this article: Medina I, Vega-Trejo R, Wallenius T, Esquerré D, León C, Perez DM, Head ML. 2020 No link between nymph and adult coloration in shield bugs: weak selection by predators. *Proc. R. Soc. B* **287**: 20201011.
<http://dx.doi.org/10.1098/rspb.2020.1011>

Received: 4 May 2020

Accepted: 4 June 2020

Subject Category:

Evolution

Subject Areas:

behaviour, ecology, evolution

Keywords:

ontogeny, colour, warning signals, avoidance, antipredator, aposematism

Author for correspondence:

Iliana Medina

e-mail: iliana.medina@unimelb.edu.au

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.5025716>.

No link between nymph and adult coloration in shield bugs: weak selection by predators

Iliana Medina¹, Regina Vega-Trejo², Thomas Wallenius³, Damien Esquerré³, Constanza León³, Daniela M. Perez³ and Megan L. Head³

¹School of BioSciences, University of Melbourne, Parkville, Victoria 3010, Australia

²Department of Zoology, Stockholm University, Stockholm, Sweden

³Division of Ecology and Evolution, Australian National University, Canberra, Australian Capital Territory 0200, Australia

IM, 0000-0002-1021-5035

Many organisms use different antipredator strategies throughout their life, but little is known about the reasons or implications of such changes. For years, it has been suggested that selection by predators should favour uniformity in local warning signals. If this is the case, we would expect high resemblance in colour across life stages in aposematic animals where young and adults share similar morphology and habitat. In this study, we used shield bugs (Hemiptera: Pentatomoidea) to test whether colour and colour diversity evolve similarly at different life stages. Since many of these bugs are considered to be aposematic, we also combined multi-species analyses with predation experiments on the cotton harlequin bug to test whether there is evidence of selection for uniformity in colour across life stages. Overall, we show that the diversity of colours used by both life stages is comparable, but adults are more cryptic than nymphs. We also demonstrate that nymphs and adults of the same species do not tend to look alike. Experiments on our model system suggest that predators can generalise among life stages that look different, and exhibit strong neophobia. Altogether, our results show no evidence of selection favouring colour similarity between adults and nymphs in this speciose clade.

1. Introduction

Warning signals are used by multiple species, throughout different life stages, to advertise unpalatability or general unprofitability [1]. Theory suggests that predation should favour uniformity in local warning signals, which facilitates predator avoidance learning [2–4]. In fact, this is the basis for the evolution of mimicry and local convergence in warning signals across distantly related taxa [5,6]. There are, however, multiple examples of variation in warning signals [7]. Most studies on variation in warning signals have focused on the apparent paradox of colour variation between populations or across individual colour morphs of the same species [4,7–10]. An understudied type of variation in warning signals is that across life stages [11]. Such variation can be especially important in insects with incomplete metamorphosis, such as bugs, where adults and nymphs share similar morphology, diet, habitat and potentially predators. If theory predicts local convergence, why would the same individual use different warning signals through its life?

Many animals exhibit dramatic colour variation as they age [12]. Ontogenetic variation can entail transitions between two different antipredator strategies, like the transition of cryptic caterpillars into warningly coloured butterflies [13]. Colour variation across life stages can also involve changes within the same type of antipredator strategy (within warning or cryptic colorations). For example, the green tree python (*Morelia viridis*) switches from cryptic yellow and red to a green colour with age, using a cryptic antipredator strategy that

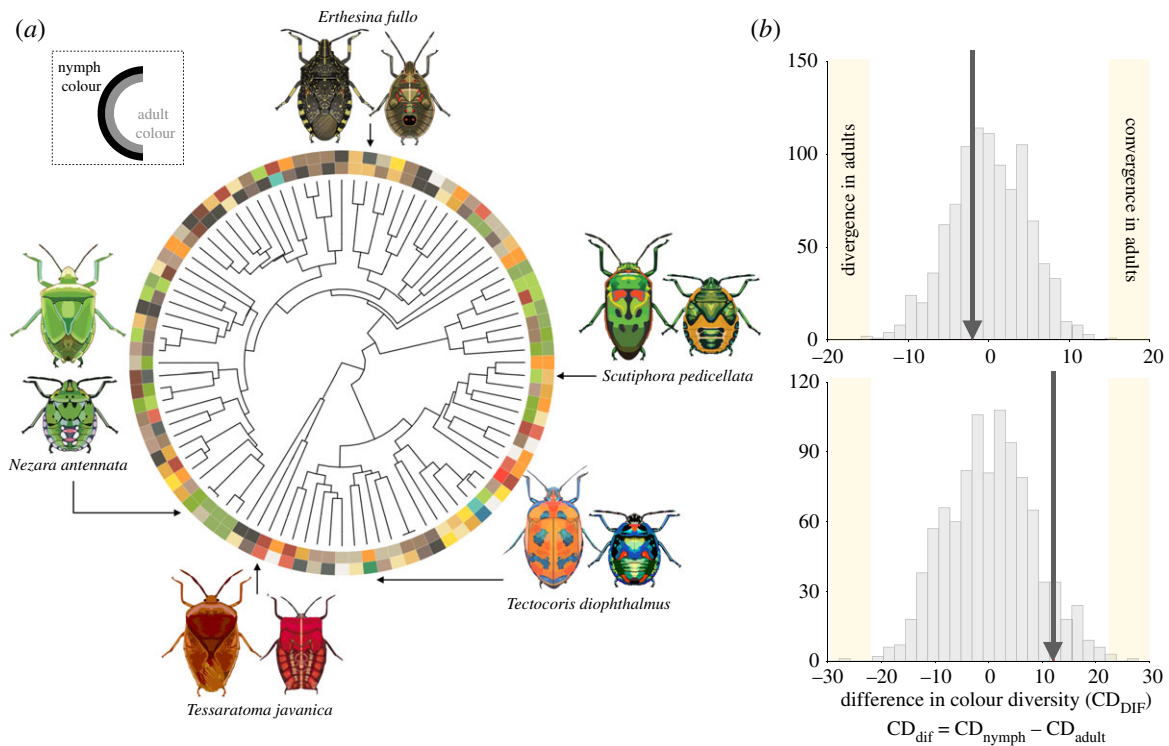


Figure 1. (a) Phylogeny of 94 spp. used in the analysis of phylogenetic signal. Colour in squares represents the main and brightest colour at each life stage (inner circle for adults, outer for nymphs). (b) Analysis of colour diversity (CD) across species for two life stages. Larger values of CD_{dif} indicate lower colour diversity among species in adults compared to nymphs (convergence in adulthood). Arrows indicate observed value in dataset. There are no significant differences in CD between nymphs and adults according to both colour distances used (type 1 (i) and type 2 (ii)). Species illustrations by D.M.P. (Online version in colour.)

matches the different habitats used by juveniles (lower vegetation) and adults (canopy) [14,15]. Moreover, the cotton harlequin bug (*Tectocoris diophthalmus*) exhibits bright coloration, as nymphs and adults, which is accompanied by defensive secretions that deter avian predators at both life stages [16,17]. Instead of maintaining the same aposematic signal, however, it goes through a dramatic colour change as it enters adulthood, despite nymphs and adults sharing the same habitat (figure 1a). Although ontogenetic changes in colour have been studied in several species across different taxa [15,18–21], there is limited understanding of this phenomenon at a broad evolutionary scale. We currently do not know how frequently and in what direction (e.g. from cryptic to aposematic) changes in antipredator coloration occur through ontogeny, and what the contribution of each life stage to the generation of colour diversity is.

There are several explanations for why variation in antipredator coloration exists across life stages. For instance, different predation pressures at different moments in life could lead to different colours between life stages [19,20]. Furthermore, some antipredator strategies might be more effective at certain life stages. Body size positively predicts the presence of warning coloration in a wide range of insects [22] and high activity levels can also be correlated with the presence of warning colours [23,24]. Both size and activity levels are traits that may differ between life stages, thus explaining variation in antipredator strategies across life. Transitions from cryptic to aposematic strategies have been reported in caterpillars and other insects and such transitions are often accompanied by changes in chemical or mechanical defences [19,25,26].

In this study, we use shield bugs (Hemiptera: Pentatomoidea) to understand how colour evolves across life stages in insects with incomplete metamorphosis, and to test whether there is evidence of selection towards similar colours

in adults and nymphs. First, we compare levels of colour diversity between species at each life stage. That is, whether colour diversity is greater in adults than in nymphs — a pattern that would be expected if, for instance, sexual selection is important in driving colour diversity. We also quantify the phylogenetic signal of colour at each life stage, and test to what degree colour evolution is coupled across life stages (i.e. whether differences between species as nymphs can predict differences as adults).

Shield bugs are known for expelling defensive secretions when disturbed, and many species advertise this defence using warning coloration [16,27,28]. Thus, our second aim is to quantify differences in colour strategies used across their lifetime, and test whether there is evidence of selection for uniformity in colour across life stages. As mentioned above, we would expect species with warning colours to have similar colorations across life stages, to take advantage of predator learning processes. We quantify colour differences between nymphs and adults in 134 species of shield bugs, and test whether colour resemblance between nymphs and adults of the same species is higher than expected by chance. Additionally, we use naive predator experiments with live shield bug prey (the cotton harlequin bug) in both the aviary and the field to understand how colour differences between life stages affect predator attack rates and learning processes. To our knowledge, this constitutes the first broad-scale study of ontogenetic variation in colour-related strategies and its ecological implications.

2. Methods

(a) Study system

Shield bugs (Hemiptera: Pentatomoidea), like the cotton harlequin bug, are a speciose (greater than 7000 spp.) group of insects with

incomplete metamorphosis. Adult and nymph shield bugs generally share similar morphology, diet, microhabitat and thus likely share similar predation pressures. In fact, many species from the family Scutelleridae (jewel bugs) are known to exhibit maternal care, and so adults and nymphs are found in close proximity [29,30]. A distinctive trait of shield bugs is the presence of defensive secretions in both adults and nymphs to deter predators [16,27,28,31].

We use as a model species the cotton harlequin bug (*Tectocoris diopthalmus*), which is a common scutellerid bug in Australia, known to be aposematic to birds, but not invertebrates [17,32]. The nymphs are blue with red markings, and the adults can be either orange with blue markings or blue with red markings. Females almost exclusively exhibit the orange coloration, while males can exhibit either, and are more likely to be blue in colder regions [33].

(b) Colour data collection for broad-scale analyses

We collected published photographs of 134 species of the superfamily Pentatomoidea (268 photos), with focus on the most colourful and speciose families (Pentatomidae and Scutelleridae). Colour is not well preserved in museum specimens of bugs, and the number of species with collected nymphs is extremely low, hence we could not use spectral measurements or standardized photographs. We searched in Web of Science for scientific articles that contained the words ‘nymph*’, ‘larvae’, ‘ninja’ plus either ‘pentatomoidea’, ‘scutelleridae’ and/or ‘pentatomidae’. From all the articles found (587 in total), we only used articles that had photographs and/or information for both juvenile and adult stages. We chose good quality photographs of the final nymphal stage (in most cases colours were found to be similar across all nymphal stages) and adults. When photos were not available in the articles, we looked online for available photographs and chose a good quality photograph that matched the colour description given in a published article.

(c) Extraction of colour measurements

For each of the 268 photographs obtained, we measured standard RGB values (566 colours, electronic supplementary material, data file) using the software Digital Colour Meter. We chose regions of the photograph that represented the different colours present in the shield bug. We did this manually to ensure that the RGB measurements were collected from patches where illumination was appropriate (e.g. no shadows or flash glare) and to guarantee that the colour measured matched the general perception of colour and was not an artefact of the photograph. We sampled between one and four different coloured regions per photograph, depending on the number of different colours identified on the organism. All data extraction was done by the same person (I.M.) and colour extraction for all the photographs was repeated three independent times (total measures: 1698) to check for repeatability of results. We also assigned each colour measured into one of four area categories: (i) primary colour (occupies more than 80% of the animal), (ii) primary colour but shared with another primary colour (each occupies between 30 and 50% of the animal), (iii) secondary colour (10–30%), and (iv) tertiary colour occupying less than 10% of the body of the animal (e.g. small spots). We used this procedure rather than quantifying colour area because photographs were taken from different angles. Given that the photographs we used differed in illumination, quality and angle, slight colour differences are probably not biologically meaningful. For this reason, we generated colour clusters to reclassify the colours sampled into broader colour categories (see details and validation in electronic supplementary material, figures S1 and S2). This reclassification is more objective than manually assigning colour categories to each colour and is more conservative than using raw RGB values from non-standardized photographs.

The colour clusters obtained were used to calculate colour distances between different species (within each life stage) and between nymphs and adults (within species) using two different measures of colour distance that take into account not only the different colours in each bug, but also the area occupied by each colour (details in electronic supplementary material). We refer to both distance types as colour distance type 1 (Earth Mover’s distance, a distance between probability distributions) and type 2 (equivalent to a Euclidean distance with six dimensions). We also used the extracted colour clusters to calculate the colour contrast between each organism and natural backgrounds, by calculating the colour distance between each individual and six different colour clusters that represent different types of natural backgrounds. These six clusters were obtained by sampling all the available natural backgrounds in the set of photographs (electronic supplementary material, figure S3). This contrast measure was used to classify coloration as contrasting, cryptic or ambiguous, based on the bimodal distribution of the average contrast against green and brown backgrounds (contrasting > 0.4, ambiguous = 0.4–0.3, cryptic < 0.3). This classification was only used to calculate differences between nymphs and adults within the contrasting and cryptic categories, where colour distances between nymphs and adults would be expected to be lower in species that are contrasting compared to those that are cryptic (because the former are expected to be under selection for uniformity).

(d) Phylogenetic reconstruction

We compiled 14 mitochondrial loci available on GenBank from species of Pentatomoidea to produce an alignment of 94 taxa plus *Urolabida menghaiensis* as an outgroup [34]. For some species, we had phenotypic but not molecular data, so we used a replacement sample from the same genus (only if this was the only species sampled for that genus) to complete our molecular alignment. If two or more species from the same genus had no molecular information, then only one species was included as replacement for the whole genus. Details on the molecular sampling and phylogenetic analyses can be found in the electronic supplementary material and table S1.

(e) Exploration of broad patterns of colour evolution

Using the colour information extracted, we asked three questions to explore how colour evolved at each life stage. First, we tested which life stage had a higher phylogenetic signal in colour, and quantified the phylogenetic signal of colour at each life stage using the multivariate version of Blomberg’s *K* in the geomorph R package [35], across 2500 possible phylogenetic trees extracted from the posterior distribution of our phylogenetic analyses. This measure can take into account multiple dimensions, so we considered information for the two main colours (and their RGB values) in each organism, following Medina *et al.* [13]. If there was only one main colour in the organism, then the colour was repeated twice in the array. Next, we tested whether the colour similarity (type 2 distance) between species in younger stages could predict colour similarity between species in adult stages following Sherratt *et al.* [36], and hence there is evidence that colour is coupled across the metamorphic boundary. To test for significance, we performed a Mantel test in the R package *picante* using the same colour array described for the calculation of phylogenetic signal (six dimensions, two colours) and a permutation of 10 000 iterations [37]. Significant effects indicate that colour distances between species as nymphs can predict colour distances between adults.

Second, we also compared colour diversity between life stages by calculating colour distances between all species within each life stage (using type 1 and type 2 colour distance measures, referred to as CD: colour diversity) and then calculating the difference in CD values between nymphs and adults ($CD_{dif} = CD_{nymph} - CD_{adult}$). Lower values of CD_{dif} indicate higher colour diversity in adults

compared to nymphs, which would correspond to ontogenetic divergence. We compared this value against a null distribution of DC_{dif} values that was generated by randomly assigning each organism measured to either life stage (1000 randomisations), following Esquerré *et al.* [38].

Third, to test whether nymphs and adults from the same species tend to look alike, we tested whether the actual distribution of colour distances between nymphs and adults is smaller than what would be expected by chance. To do this, we first calculated the distribution of colour distances between nymphs and adults using the two types of colour distance (described in electronic supplementary material). Then, we generated 1000 random distributions of colour distances by shuffling the identity of the nymphs and randomly assigning them to the adults and recalculating the colour distances. We compared the real distribution of colour distances to each of the random distributions using a Bayesian approach (details in electronic supplementary material). If real colour distances are smaller than randomly calculated colour distances (e.g. difference below zero), then this would be evidence that nymphs and adults of the same species have a tendency to look alike.

(f) Generalization experiment in the aviary

To test whether naive predators could generalize avoidance between nymph and adult cotton harlequin bugs (*T. diophtalmus*), we obtained 60 chicks (*Gallus gallus*, two weeks old) from a commercial hatchery (further details on acclimation period and experimental set-up in electronic supplementary material) in February and March 2018. For logistical reasons related to the size of the aviary, the experiment was repeated in three different weeks (i.e. batches), and this is taken into account in the statistical analyses. We used female bugs of the same colour (orange morph) for this experiment and the field experiment, because we did not have enough males. At three weeks of age, naive chicks were trained to avoid either adult or nymphal bugs and then trained chicks were used in a generalization trial where they were presented with the opposite life stage to what they had been trained on. During training, naive chicks were placed in an experimental arena and presented individually with a treatment insect (either an adult or a nymph). The insect was presented inside a Petri dish with a green background, generated from photographs of leaves taken of the bug's host plant. Before each training session, birds were deprived of food for an hour. Chicks were individually trained to avoid the treatment insect in seven training sessions, each session lasting 1 min. After each training session, we presented each chick with a mealworm and corn kernel to ensure they were motivated to eat (which they always were, details in electronic supplementary material). An attack was recorded as occurring if the beak of the bird touched the insect. After each chick had completed at least three consecutive training sessions without attacking the insect, we assumed that avoidance was learned. Only chicks that had reached this criterion went into the second phase of the experiment of generalization, following Rönkä *et al.* [39] (chicks included: trained with nymph $n = 17$, adults $n = 19$, chicks excluded because they did not learn: five trained with nymphs, two with adults). Three hours after the last training session, we performed one generalization test, where the chick was presented with a bug of the opposite life stage from that which they were trained with. That is, if a chick had been trained to avoid adults, then they were presented with nymphs in the generalization test (and vice versa). The generalization test was performed in the same way as the training sessions. For all training sessions and the generalization trial, we recorded attack/no attack and calculated attack latencies from videos.

(g) Field predation experiment

To explore how wild predators respond to the warning signals of the cotton harlequin bug when they see them for the first time,

we presented live bugs to wild naive white-winged choughs (*Corcorax melanorhamphos*) in Canberra, Australia, between April and May 2018. Cotton harlequin bugs are not distributed naturally in Canberra, and to the best of our knowledge, there are no common species that look alike in Canberra, so they represent a novel stimulus to the birds used. Choughs are insectivorous passerines that co-occur naturally with cotton harlequin bugs in their northern range (New South Wales and Queensland), and are thus potential predators of this species. To measure attack rates by choughs, we used a banded population of more than 100 individuals. This ensured that the choughs used in the experiments were naive to the stimulus and individual choughs were not used more than once. Given that choughs live in social groups of 4–20 individuals [40], we set up a hexagon with six bugs of the same type tied to a nail on each on the corners of the hexagon. For each group of choughs (i.e. each hexagon), we used either orange females (six groups with 33 choughs in total entered hexagon), nymphs (eight groups, 48 choughs) or commercially obtained crickets (as positive control, five groups, 48 choughs). Chough groups were attracted to the hexagon using a small amount of cheese as this banded population has been trained to eat cheese for other studies. For all experiments, three researchers observed with binoculars from different angles at 3 m away from the hexagon. We recorded the band number of the choughs in the experiment and counted the number of attacks that occurred. We considered attacks only those instances where the beak of the bird touched the insect. Observations started when the first bird entered the hexagon and lasted 5 min.

(h) Statistical analyses of aviary and field experiments

To test whether aviary chicks that had learned to avoid either nymphs or adults generalized this avoidance to the other life stage, we used a binary generalized linear model (GLM). Since we wanted to test whether learning with one prey type could decrease the probability of attacking a novel prey, we compared the probability of attack during the first training session (when chicks were first presented with the treatment bug) with the probability of attack during the generalization session (when chicks had previous experience with the opposite life stage). The response variable in the model was binary (i.e. whether an attack occurred or not) and the predictor variable was the level of experience (first training session or generalization). We also included 'batch' number as a predictor in the models to account for differences in the period which the experiment was performed. We did not include chick ID because the groups of chicks being compared were different. Besides the analysis of the binary variable of attack/not attack, we also used information on attack latencies to test for generalization in 83/85 videos (in two experiments, the camera failed). For this, we used a linear model with a continuous response variable (time to attack). We used logit transformation on the time to attack because, given the nature of our experiment, the time was bounded between 0 and 60 s. We used the level of experience of the chick (first training session or generalization) and the batch number as predictors. In this analysis, 'no attack' was recorded as a latency time of 60 s (the maximum time in the trial). If chicks generalize avoidance between life stages, then we would expect the probability of attack to decrease, and the latency of attack to increase, when the chicks had previous experience with an insect (demonstrating increased aversion). We ran the models using lme4 and used the DHARMA R package [41] to test for convergence in the model and the distribution of the scaled residuals.

To test whether naive predators (choughs) in the wild were more likely to attack nymphs or adult females, we used a GLMM with a binomial distribution, where each of the six bugs presented to a group had an attack value of 1 or 0 (i.e. whether an attack occurred or not). We included in the model the size of the bird group to which the insect was presented and the type of

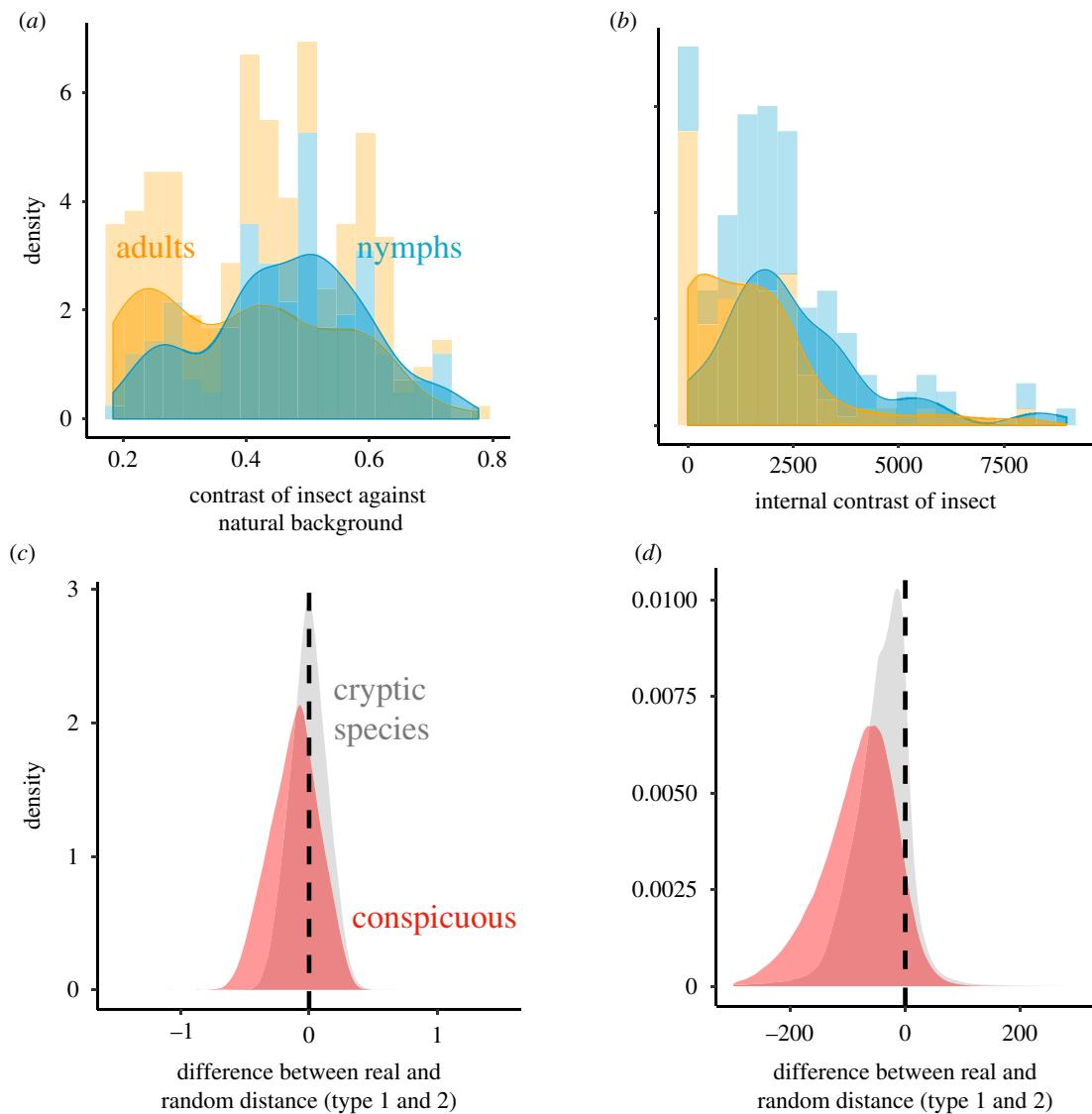


Figure 2. (a,b) Histogram and density plots of (a) colour contrast against natural backgrounds and (b) internal contrast for nymphs and adults. Nymphs are more contrasting against natural backgrounds and have higher internal contrast. (c,d) Colour distances between nymphs and adults of the same species are not smaller than expected by chance, for species classified as conspicuous (red) or cryptic (grey). The same pattern is found for the two colour distances used in the analyses (type 1, c, and 2, d). A value of zero indicates that the difference in colour between a nymph and an adult from a random species is the same as the difference between that nymph and the adult of the same species. (Online version in colour.)

insect (i.e. nymph bug, adult bug or cricket) as fixed predictors. Since each group was presented with six bugs, we also included the ID of the chough group as a random factor.

3. Results

(a) Broad-scale patterns of colour evolution

Adult and nymph coloration showed similar magnitudes of phylogenetic signal and this was consistent across the three different photograph measures taken: overall Blomberg's multivariate K (K_{mult}) was low (between 0.023 and 0.275, electronic supplementary material, table S2; figure 1a). Among species, colour distances (not colour itself) between nymphs significantly predicted colour distances between adults (average Mantel $r=0.10$, $p<0.05$, electronic supplementary material, table S3), meaning that two similarly coloured nymphs would become two adults with similar coloration. Nymphs and adults showed similar levels of colour diversity across species, and there was no sign of ontogenetic colour

convergence or divergence (CD_{dif} not different from null distribution, electronic supplementary material, table S4, $p>0.05$; figure 1b).

Colour distances between nymphs and adults of the same species were not smaller than expected by chance, and the difference in real and random colour distances between nymphs and adults of the same species was not significantly different from zero (for cryptic species—HPD (highest posterior density) interval: -0.28 to 0.27 and -145.90 to 46.08 , for contrasting species -0.47 to 0.25 and -219.54 to 19.49 , figure 2c,d). The colour distance between the adult and nymph cotton harlequin bugs (the species used in our experiments), was not uncommon in our dataset, and between 22 and 26 spp. in our sample (depending on the distance type used) presented similar or higher levels of colour differences between nymphs and adults.

Nymphs more frequently showed high levels of contrast against natural backgrounds and greater internal contrast compared to adults (species with background contrast greater than 0.4: 64 adults and 92 nymphs, figure 2a,b). The most

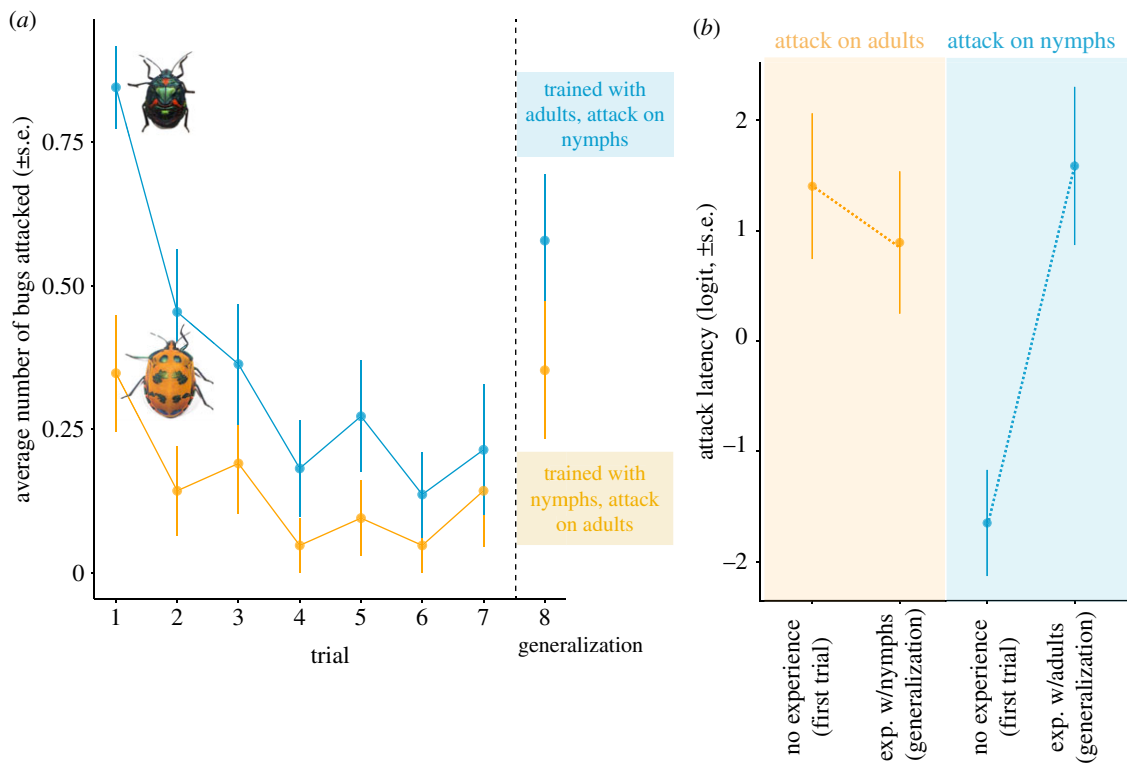


Figure 3. Aviary experiment with naive chicks. (a) In seven trials, there was a significant decrease in the attack rate of both adults (lower line; orange) and nymphs (blue). There were lower attack rates in the generalization trial but only when chicks were initially trained to avoid adults (blue colour in generalization column), which were less likely to attack nymphs. (b) Attack latency during first trials and generalization trials. There is a significant increase in nymph attack latency when chicks have been previously trained to avoid adults (blue). Insect photos by D.E. (Online version in colour.)

frequent colour strategy was conspicuous (i.e. contrasting against the background) as both adults and nymphs (56 species). The most common transition was from being a conspicuous nymph to a cryptic adult (25 spp.) and the least common transition was from cryptic nymphs to conspicuous adults (4 spp.).

(b) Aviary predation experiments

Nymphs of the cotton harlequin bug were attacked significantly more often than adults in the first trial (84.6% versus 34.7%, $Z = 3.34$, $p < 0.001$, figure 3a). Chicks significantly decreased the number of attacks for both life stages within six trials (nymphs $Z = -4.72$, $p < 0.001$, adults $Z = -2.48$, $p = 0.01$). There was a marginal difference between the number of nymph attacks for chicks with no experience with *Tectocoris* versus chicks that had previous experience with an adult ($Z = -1.932$, $p = 0.053$, nymph attacks first trial: 84.6%, nymph attacks after experience with adults: 57.9%, figure 3a). Previous experience with nymphs, however, did not decrease attack rates of adults ($Z = -0.018$, $p = 0.986$, adult attacks first trial: 34.7%, attacks after experience with nymphs: 35.2%). The analysis of latency times revealed a similar pattern where attack latency towards nymphs increased significantly in the group of chicks that had previous experience with adults (t -value = 3.199, $p = 0.003$, figure 3b). In those cases, where there was a nymph attack after previous experience with adults (11/19), attack latency was on average 14 ± 16 s, compared to attack latency towards nymphs of 3 ± 1 s when chicks had no previous experience (22/26, t -value = 2.627, $p = 0.013$). There was no difference in latency to attack adult bugs between chicks with or without previous experience with nymphs (t -value = 0.253, $p = 0.802$).

(c) Field predation experiments

We were able to locate 22 different groups of choughs with a mean group size of 6.73 individuals. In total, we observed 10 attacks on the bugs (11% of the 84 insect presentations, four adults, six nymphs) and 30 attacks (100%) on the cricket presentations. Four attacks on bugs (40%) were carried out by young individuals (less than 2 years). There was no difference in the probability of attack on nymphs or adults (Z -value = -0.191 , $p = 0.848$) and chough group size had a marginal effect on the attack rate, with larger groups attacking more often (Z -value = 0.427, $p = 0.053$). It is important to mention that given the low overall attack rate, we had only 15% chance of detecting significant differences in attack rates between nymphs and adults (four versus six attacks) at a significance level of 0.05.

4. Discussion

Ontogenetic variation in antipredator coloration is a common phenomenon that has received little attention. Changes in warning signals across life stages, in particular, have been rarely studied compared to other types of variation in warning signals (e.g. between populations or polymorphisms). Our broad-scale analyses indicate that colour in nymphal and adult stages of shield bugs is equally diverse among species, and both life stages have similar levels of phylogenetic signal in colour. This is opposite to the trend shown in butterflies, where adults tend to be more colourful and have lower phylogenetic signal in colour than caterpillars [12,13]. Despite our findings suggesting similar paths in colour evolution across life stages in shield bugs, our results also suggest that there is no selection for adults and nymphs to have similar coloration. First, we show that nymph and adult coloration within species

is not more similar than expected by chance. Second, we show that predators have flexible learning rules and neophobia, which may result in relaxed selection for uniformity in warning signals.

In many species, the transition into adulthood is accompanied by novel selective pressures that may impact the evolution of coloration. For instance, numerous butterfly species are sexually dimorphic and sexual selection is an important factor driving colour diversity in butterflies but not caterpillars [12,42]. Likewise, damselflies present ontogenetic colour changes when they become sexually active [18]. Conversely, in crabs, there is convergence in adult coloration towards dark green, which increases camouflage in a wide variety of habitats [20], suggesting strong selection for camouflage in adulthood that is not present in juvenile stages. Our results show mixed evidence for differential selective pressures on nymph and adult shield bug colour. We show that having conspicuous coloration is more common in nymphs than adults, which could suggest that there is greater selection for nymphs to have warning signals. These ontogenetic differences in antipredator strategies, however, have had no effect on colour diversity among species, and nymphs are as diverse in colour among species as adults.

It is puzzling that adults, which are usually larger than nymphs, tend to be more cryptic. Previous studies have shown an association between body size and conspicuousness, where larger organisms are more likely to exhibit warning coloration [13,22]. Warning signals are expected to be more common in larger organisms because larger individuals are inherently less cryptic (because they are more visible due to their size) and likely have higher levels of chemical defences [43]. Adult shield bugs are atypical – they exhibit no warning colours despite being larger (compared to the nymphs) and producing a strong and distinct defensive secretion. Endler & Mappes [44] previously pointed out that we do not fully understand why this is the case. Future studies could explore whether transition to less conspicuous colours in adulthood is accompanied by a decrease in the potency of chemical compounds or a decrease in mobility, which is usually linked to cryptic strategies [23]. Alternatively, there might be costs in colour production or weaker selection in adults to advertise their toxicity. Conspicuous coloration should be adaptive only if it facilitates avoidance learning, otherwise strong chemical defences combined with crypsis could be a more stable strategy [45]. Adult shield bugs constitute an attractive model system to explore when toxicity does or does not lead to the evolution warning coloration.

The similar levels of colour diversity seen between adult and nymphs in our study, and the similar levels of phylogenetic signal, suggest that sexual selection is unlikely to have led to the great colour diversity in this clade, which coincides with the lack of literature on this topic. To our knowledge, there is no clear evidence that shield bugs use colour as sexual signals and, in general, vision is not central in sexual selection in non-predatory heteropterans that live in dense vegetation; instead, these species use body size information and vibrational cues during courtship to identify potential mates [46–48].

Interspecific colour distances between nymphs can predict colour distances between adults, and similar looking nymphs will probably become similar looking adults (suggesting some coupling across the metamorphic boundary or ontogenetic constraints). Nymphs, however, are not significantly more

similar to their adults than to other adults in our dataset (figure 2*c,d*). This finding suggests that selection towards uniformity in colour between adults and nymphs is not strong, not even in species that are considered conspicuous and likely to use colour as a warning signal. This contrasts with what would be expected if predators were selecting for similarity between signals that occur in sympatry, which explains the evolution of mimicry between many distantly related species [6,49,50]. Our results from the broad-scale analyses contrast also with findings from field experiments, where it has been shown that novel or rare morphs have higher predation rates, suggesting that predators select for uniformity in local warning signals [5,10,51].

Our aviary and field results support the findings from our broad-scale analyses. We found evidence in line with the idea that predators can generalize avoidance in at least one direction, and predators that have previous experience with adults are less inclined to attack nymphs when they see them for the first time. In a natural setting, this would mean that before eggs hatch those predators that have had experience with adults may not even try to attack the recently hatched nymphs. It is possible that general colour features facilitate generalization of avoidance between nymphs and adults. It has been shown in different experiments that attributes such as brightness and internal contrast can allow predators to generalize among signals [52–54]. Moreover, given that we used live prey, the odour of both nymphs and adults may have facilitated generalization among these different phenotypes as well, although differences in the volatile compounds of nymphs and adults have been reported in this species previously [16].

An alternative explanation to the apparent ‘generalization’ in our aviary experiment could be neophobia. Negative experiences could result in increased neophobia regardless of the stimuli involved [55]. The negative experiences with adults could have modified foraging decisions in the chicks, decreasing attack rates of nymphs without involving generalization processes. Bad experiences with adults may have involved adult unpalatability due to chemical compounds, but also unpalatability/wariness from the very hard exoskeleton that adults have [56]. Many predators are quite conservative in their food intake and neophobia is suggested as a critical mechanism in the evolution of warning signals [57]. Our experimental set-up does not allow us to discriminate between these two scenarios (generalization or increased neophobia after previous experience), or their relative contribution to the low attack rates.

Our field and aviary experiments also showed that predators can have high natural levels of neophobia. Adults had low attack rates in the aviary experiment, and naive choughs (wild subjects) rarely attacked the cotton harlequin bugs. Either of the mechanisms invoked above (initial neophobia, generalization or increased neophobia due to experience with adults) are likely to lead to weak selection from predators towards signal uniformity across life stages. It is worth pointing out, however, that the capacity for generalization and neophobia could vary among predator species, so it would be interesting to replicate this experiment using other insect/predator systems. Furthermore, although we think it is improbable (due to shared habitat and similarity in shape and size), we cannot completely reject the possibility that adults and nymphs are attacked by different sets of predators in the wild. If this were the case, strong selection for

divergence, rather than weak selection for convergence, could explain the high ontogenetic variation in colour in the clade.

5. Conclusion

Our multi-species analyses of shield bugs indicate that colour is equally diverse among nymphs and adults, but that different antipredator strategies and colour patterns are being used by different life stages. Ontogenetic variation in colour seems to be very common in the clade – at least in our sample focused on the families Scutelleridae and Pentatomidae. We found that the colour of nymphs and adults is not more similar than expected by chance. Future field and experimental studies could explore why nymphs tend to be more colourful and conspicuous in these speciose clades, and which selection pressures are responsible for divergence in colour at both life stages. Our results also show that the common expectation for uniformity in warning signals is not observed at a broad scale, nor should it be predicted based on our experiments with predators [7,50]. In other words, the premise that predators select for uniformity in local warning signals is not supported in jewel bugs, and relaxed selection could allow variation in signals across life stages in this clade [3]. The amazing examples of convergence in Müllerian and Batesian

mimicry that we know might be rarer than we think. In the recent years, we have become more aware of how common imperfect mimicry is [58], how flexible predator learning processes can actually be [59,60] and how common variation in warning signals within species is [7]. Our study adds to this list, demonstrating that ontogenetic uniformity in warning signals might be the exception rather than the rule.

Ethics. This research adheres to the ASAB/ABS guidelines for the use of animals in research and was approved by the Animal Experimentation Ethics Committee at the Australian National University under permits A2017/42 and A2018/03.

Data accessibility. Data and code have been uploaded to the Dryad Digital Repository: <https://doi.org/10.5061/dryad.w3r2280n7> [61].

Competing interests. We declare we have no competing interests.

Funding. This work was supported by a McKenzie Research Fellowship from University of Melbourne to I.M., a British Ecological Society grant to I.M. and M.L.H. and an ARC Future Fellowship to M.L.H.

Acknowledgements. We would like to thank Devi Stuart-Fox, John Endler and Hannah Rowland for comments and discussion. I.M. would like to thank Daniele Silvestro and Tobias Andermann for help with the implementation of the Bayesian analysis. We would also like to thank Scott Fabricant and Emily Burdfield-Steel for help finding collecting sites and two anonymous reviewers for constructive comments on previous versions of the manuscript.

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