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Despite the fact their coloration functions as an aposematic signal, and is thus expected to be under stabilizing selection, hibiscus harlequin bugs (*Tectocoris diophthalmus*) show an impressive level of variation in their iridescent coloration both within and between populations. To date the heritability of coloration in this species remains unknown. Here we focus on a single population in New South Wales (the southern part of this species' Australian range), with the greatest colour variation. We reared full-sib families of known pedigree in the laboratory and analysed the extent of iridescent coloration at adulthood. We then looked for evidence of heritability, condition dependence and antagonistic sexual selection acting on colour in this species. We found significant heritability in the extent of iridescent coloration for both sexes, as well as in development time and body size, but no evidence that condition dependence played a role in the determination of adult coloration. There was, however, a sex by genotype interaction for iridescent cover, in the form of a negative intersexual genetic correlation: in families where sons had high iridescent cover the daughters had low, and vice versa. Our results suggest that different selective pressures may act on coloration in males and females of this species.

ADDITIONAL KEYWORDS: aposematism – coloration – Hemiptera – heritability – Heteroptera – warning coloration.

INTRODUCTION

Animal coloration has many functions. It may be used to protect from harmful UV radiation (Ortonne, 2002), to regulate body temperature (Hegna *et al.*, 2013), attract a mate (Kemp, 2007), blend in (Stevens & Merilaita, 2009) or, in the case of warning colours, stand out (Gamberale-Stille & Tullberg, 1999; Rojas *et al.*, 2015). Many defended organisms; i.e. those that possess chemical, physical or even behavioural features that render them unprofitable to predators, seek to advertise their defended status using so-called 'warning' coloration. This typically consists of highly conspicuous colours and patterns thought to allow predators to more easily learn and subsequently avoid unprofitable prey, presumably to the benefit of both interactants (Endler, 1988).

The association of signal and defence is known as aposematism, and is widespread in nature. Due to its

dependence on predators being able to easily recognize defended prey, aposematic signals are expected to be under stabilizing selection and to therefore exhibit reduced variation (Poulton, 1890; Borer *et al.*, 2010). Species with colour-based aposematic signals nevertheless show considerable variation (Rojas & Endler, 2013). In a recent review, Briolat *et al.* (2019) highlighted the need to consider both the genetic underpinnings of signal production and the variety of potential selection pressures at play in order to understand how such variation can persist (Briolat *et al.*, 2019). One species for which variable aposematic coloration has been investigated from both predatory and environmental perspectives is the hibiscus harlequin bug (also known as the cotton harlequin bug), *Tectocoris diophthalmus* (Heteroptera: Scutelleridae), which is an emerging model organism for the study of such variation.

Hibiscus harlequin bugs couple chemical defence (Staddon *et al.*, 1987) with highly conspicuous body coloration. In adults, the latter consists of a mosaic

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of pigmentary orange/red (produced by erythropterin) and iridescent blue/green markings (a form of structural coloration) (Fabricant *et al.*, 2013). This colour scheme is known to trigger avoidance learning in birds (Fabricant & Smith, 2014). Despite this, there is great variation in colour pattern, both among sexes (Ballard & Holdaway, 1926; see Fig. 1), and between and within populations (Fabricant *et al.*, 2018). The extent of structurally-coloured markings can vary from entirely absent to covering virtually the entire dorsal surface (Fig. 1). This variation appears continuous in populations from the southernmost reaches of its Australian range, whereas northern populations are often either entirely blue or orange, with few intermediates (Fabricant *et al.*, 2018). This species therefore exemplifies the question: if coloration is strongly selected for its salience and memorability as an aposematic warning signal, then why does so much variation persist?

There are a number of possible explanations for lack of convergence on a common signal. The first is that the variation seen is entirely environmentally produced, with no heritable component. One mechanism by which coloration could be influenced by environment is through resource availability. Although the costs of accumulating the orange, erythropterin, pigment are thought to be negligible (Harmsen, 1966), the condition-dependence of coloration has not been studied in this species. The iridescent patches in particular are formed from layers of melanin, which plays an important role in insect immune function (Nakhleh *et al.*, 2017). Therefore trade-offs may exist between coloration and immune function. Another mechanism for environmental effects on coloration is through temperature, and indeed *T. diophthalmus* do show colour plasticity in response to temperature during development [as is common in many insect species, see (Sibilia *et al.*, 2018) for one example].
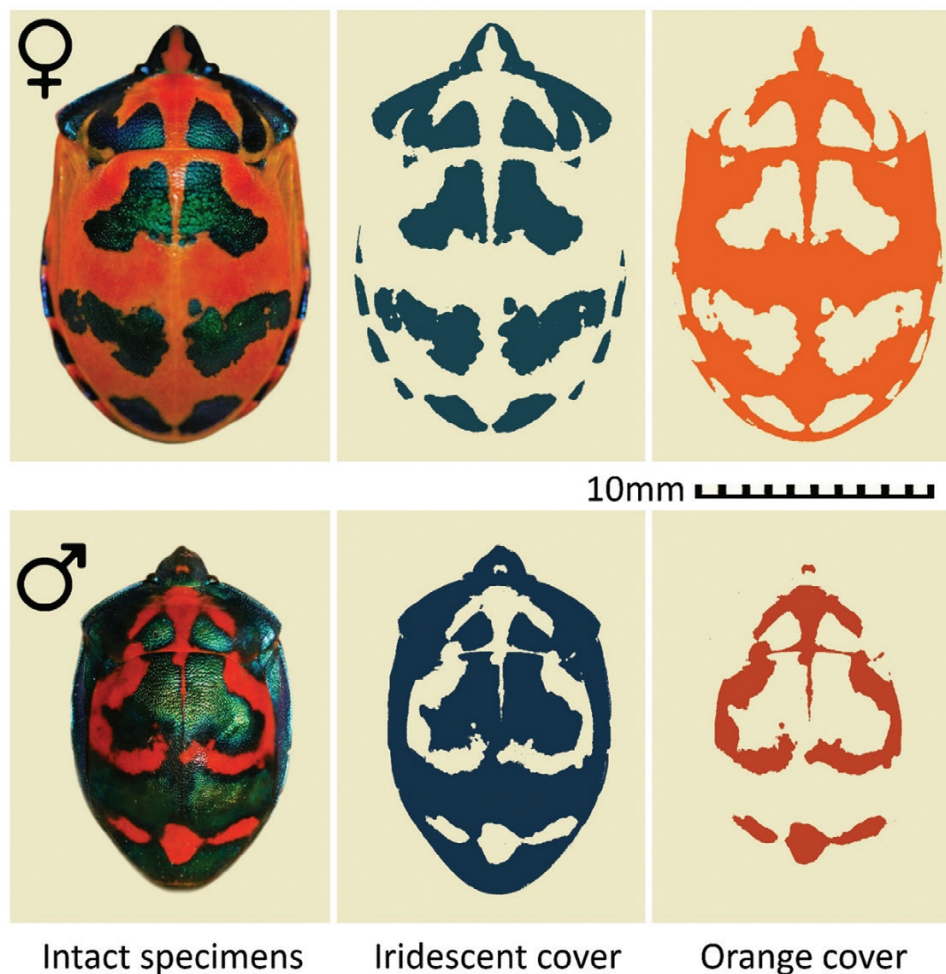
Figure 1. Images of intact female and male *T. diophthalmus* specimens along with "exploded" images showing the total region of each dorsal surface that would, by our methods, be classified as iridescent vs. orange coloration.

Once they moult into adulthood, coloration is fixed, with warmer conditions resulting in a more orange-dominated colour pattern. However, the same study also found a clear signal of population of origin, suggesting that colour variation is at least partly heritable. The role of genetic factors is further supported by the different underlying distributions of colour variation seen across different geographical regions, which may be underpinned by gene-by-environment interactions. Relevant to this, increased rearing temperatures cause bugs from tropical northern Australia to "switch" from predominantly blue to all orange, whereas plasticity in bugs from the temperate southern population is much more continuous (Fabricant *et al.*, 2018).

If coloration does have a genetic component then disruptive selection by different classes of predators could be maintaining the observed variation. Avian predators (both captive and wild) have been shown to avoid the bugs, and there is evidence that the iridescence itself acts as a warning signal and that a mix of orange and iridescent patches may provide better protection against birds than orange alone (Fabricant *et al.*, 2014). Mantid predators however, appear unaffected by the bugs' chemical defences (Fabricant & Smith, 2014). In addition, the monochromatic visual system of mantids means that orange bugs appear cryptic to them, and experiments have shown that bugs with blue patches were more likely to fall prey to mantids in a laboratory setting (Fabricant & Herberstein, 2014). Thus these two predator types may select for different colour patterns.

A third possibility is intra-locus sexual conflict (Parker, 1979; Bonduriansky & Chenoweth, 2009). Hibiscus harlequin bugs show a degree of sexual dimorphism, males tend to have larger blue patches than females. However, there is a significant overlap between the two sexes (Fabricant *et al.*, 2018). It could be that selection favours a different optimal colour phenotype in each sex. Although there is currently no evidence either way for a role of sexual selection in these insects [due partly to difficulties with captive breeding; (see Keller, 2012)], adults of two sexes do differ markedly in behaviour and likely exposure to predators as well as other environmental variables (e.g. temperature). Female bugs lay and guard egg clutches (Giffney & Kemp, 2014, 2016) for the entirety of egg development (approximately 17 days; Dodd, 1904; Ballard & Holdaway, 1926). As they typically oviposit on the outer stems of their host, this may expose them to greater predation risk [although see (Giffney & Kemp, 2014)], as well as to heat, UV radiation and desiccation risk. Males are by contrast more active and mobile in their mate-searching behaviours, and may have a shorter lifespan (pers. obs.). Finally, measures of toxicity using *Daphnia* suggest that males are more chemically defended relative to body weight than females (Medina *et al.*, 2020). Females are also typically larger than males, which may influence all these processes.

If the mechanisms of colour production and expression are controlled by the same genes in males and females, different phenotypic trait optima for the two may result in intra-locus sexual conflict. Although this could be resolved to some degree via the evolution of sexual dimorphism (Fisher, 1958; Lande, 1980), complete divergence may not be achieved due to the constraints of genetic architecture (Lande, 1980; Bonduriansky & Chenoweth, 2009). In particular, if colour pattern has a complex polygenic basis then constraints upon the independent evolution of the sexes may explain the extreme levels of variation observed in this species.

To distinguish between the potential mechanisms for colour variation in this species we need to know: (1) the extent of heritability of the colour pattern; (2) the extent to which variation is condition-dependent; and (3) the magnitude of intersexual correlation. We sought answers to these questions by rearing wild-collected egg clutches under common-garden conditions and examining adult coloration in relation to pedigree and body size. This approach also provided some insight into the genetic architecture of sex-specific development in this species and enabled assessment of the extent to which genetic variation for adult coloration features in this architecture.

MATERIAL AND METHODS

SAMPLING POPULATION

Thirty female *T. diophthalmus* together with their egg clutches were collected from Narrabeen (*c.* 20 km north-east of Sydney), Australia, on 21–23 December, which coincides with the end of the first annual breeding cohort at this location. The species is highly abundant throughout the region where individuals of all life stages can be found upon *Lagunaria patersonia* (Norfolk Island hibiscus).

HUSBANDRY PROTOCOLS

Females were retained with their clutches until the day of hatching, when they were removed and frozen at -30 °C. Hatchlings were subsequently reared in full-sibling groups of $N = 2$–8, spread among multiple 750 mL plastic cups, under constant conditions of 25.0 ± 1.0 °C and 14h:10h L:D photoperiod. These conditions approximately mimic those experienced during the summer months in Sydney, but without the typical daily fluctuations in temperature. They

were supplied every 2–3 days with fresh cuttings (containing fruit) of *L. patersonia* collected from the field-site.

DATA COLLECTION

We recorded juvenile development time as the number of days from egg hatching until the final (adult) moult. The reciprocal was calculated to yield developmental rate in units of d^{-1}, which expresses the proportion of development completed per day. All individuals were sexed and photographed upon reaching adulthood, with a subset weighed (to the nearest 1.0 mg) at approximately 24 h post eclosion. Photographs were taken under diffuse (fluorescent) overhead lighting with bugs situated against a common neutral background using a Canon EOS400D digital camera fitted with an EF-100 mm fixed focal-length macro lens. Each image included a graduated scale.

Photographs were processed using Image J (NIH) software to measure the dorsal surface area as well as the area covered by iridescent coloration (both in mm^2). These values were then used to calculate the proportional cover of iridescence. We calculated this parameter both as a simple percentage (i.e. iridescent area/body area) and as the standardized residuals from separate-sex regressions of iridescent cover upon body area (both in mm^2; see Fig. 2). The percentage and residual measures were near-perfectly correlated in both sexes (males r = 0.975; females r = 0.985), and we used the latter—residual body area—in analysing phenotypic relationships with
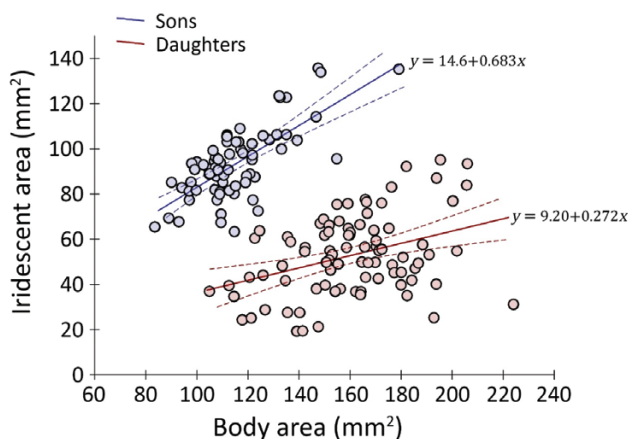
Figure 2. Overall relationships between dorsal body area and absolute iridescent cover for sons and daughters. Linear best fits are accompanied by 95% confidence bounds and described by the equations indicated. Each regression was significant (males: $F_{1,68}$ = 70.24, *P* < 0.001, r = 0.713, adjusted R2 = 0.501; females: $F_{1,84}$ = 18.28, *P* < 0.001, r = 0.370, adjusted R2 = 0.126).

body mass variables. For the main (genetic) analysis of size-relative iridescent coverage we simply used the absolute value of iridescent cover but included body area as a fixed covariate. This means that the analysis was levelled at marginal or size-corrected iridescent coverage. All conclusions were unchanged (at α < 0.05) regardless of precisely how relative iridescent cover was parametrized in these analyses.

Residual mass was calculated as the standardized residual of a linear regression of body weight (mg) upon body area (mm^2) as a proxy for body condition. Each variable was in this case natural-log transformed because the relationship between a linear variable and a volumetric variable is expected to approximate a logarithmic power function.

STATISTICAL ANALYSES

Our main analyses consisted of general linear mixed modelling (GLMM) to partition trait (co)variances and then to estimate heritabilities and genetic correlations across sexes and traits. We conducted heterogeneous variance-based correlation models that included sex as a fixed effect and family and rearing cup (nested within family) as random effects. Fixed effects were tested for significance using conditional Wald *F*-tests that were adjusted (Kenward & Roger, 1997) to respect the marginality relationships among fixed GLMM factors [for further detail see (Gilmour *et al.*, 2015)]. All reported *F*-tests for fixed GLMM effects are therefore conditional Wald *F*-tests.

We ran single trait models for our three primary dependent variables (relative iridescent coverage, body size and development time), and a multi-trait model for all three. The single trait models proved more stable and amenable to iterative model fitting procedures (Kruuk, 2004; Wilson *et al.*, 2010) which we used to derive the most parsimonious (co)variance structure for each trait. This involved first fitting the simplest (most constrained) model; that is, one with a single genetic and residual variance for all individuals regardless of sex and with an intersexual genetic correlation fixed at 1.0. The model was then re-ran in progressive steps to allow: (1) residual variances to vary across the sexes; (2) genetic variances to vary across the sexes; (3) intersexual genetic correlation (r_G) to vary from 1.0; and finally (4) to include "rearing cup" as a random variable nested within family. At each step we calculated the change in overall goodness of model fit (δLL) as twice the log-likelihood difference from each previous model. This was evaluated against the χ^2_1 critical value (Kruuk, 2004). Only changes resulting in a significant gain of model fit were incorporated, and so the procedure iteratively arrived at the most parsimonious variance structure for the data. The evaluation of δLL also provided formal significance

tests for whether variances differed among sexes (steps 1–2), for whether genetic correlations varied from 1.0 (step 3), and for the presence of any rearing cup effect (step 4). The final (most parsimonious) models were used to test fixed effects and estimate genetic parameters (H^2 and r_G).

We supplemented this approach with a multi-trait model conducted primarily to estimate genetic correlations among (absolute) iridescent cover, body size and development time. This model involved a considerably more complex design, with no fewer than 24 separate (co)variance parameters to be simultaneously estimated (six each of residual variances, residual covariances, genetic variances and genetic covariances). Successful convergence was contingent upon specifying initial parameter values as gained from the single trait solutions. Attempts to subsequently constrain the variance structure to explore model-fitting, however, created convergence problems and generated singularities in the information matrix; we therefore report observational estimates from the least constrained version of this model.

The random (sparse) GLMM solutions provided estimates of genetic variance (σ^2_G) and residual variance (σ^2_R). These were used to calculate broad-sense heritability (H^2) for each sex as:

$$H^2 = \frac{\sigma^2_G}{\sigma^2_G + \sigma^2_R}$$

We assumed that hatchlings from each brood consisted of full siblings because this will attribute components of within-family variance more conservatively with respect to their potentially shared genetic basis (i.e. assuming half-sibling relatedness would effectively double the estimates for genetic parameters relative to a half-sibling scenario). Given that the coefficient of relatedness for full-sibs is 0.5 (Falconer, 1981), we divided genetic variance components by 0.5 when estimating genetic variances and heritability.

Genetic correlations were calculated as per the standard equation based upon (co)variance estimates (Falconer, 1981; Lynch & Walsh, 1998). Intersexual genetic correlations were, for example, calculated using genetic variance estimates for males (σ^2_m) and females (σ^2_f) and the estimated genetic covariance between the sexes (σ_{mf}) according to the formula:

$$r_G = \frac{\sigma_{mf}}{\sqrt{\sigma^2_m \cdot \sigma^2_f}}$$

Simpler statistics were conducted and are reported as per convention. Means are accompanied by standard errors throughout unless otherwise indicated. Analyses were conducted using ASReml (Gilmour *et al.*, 2015) and Statistica v.7.

RESULTS

From the initial 30 clutches we reared a total of 156 bugs across 17 putatively full-sibling families, 86 (55%) of which were females. The initial collection resulted in fewer realized broods due to hatching failures and systematic infection by scelionid parasitoids (Giffney & Kemp, 2014). Size and colour data were collected from all specimens, with body weight assessed for 97 specimens in 13 families. The range of iridescent coverage for females was 5–70% of the dorsal surface covered with iridescent patches, with a mean of 34%. For males, the range was 20–96%, with a mean of 76%.

### CONDITION-DEPENDENCE OF COLOUR CONSTITUTION

Relative (size-controlled) iridescent coverage was categorically unrelated to residual mass in males ($r = -0.141$, $N = 51$, $P = 0.325$) and females ($r = -0.005$, $N = 54$, $P = 0.969$). There was likewise no relationship between relative iridescent coverage and raw body mass (males: $r = -0.052$, $P = 0.717$; females: $r = -0.105$, $P = 0.448$). This indicates the absence of phenotypic condition-dependence for colour pattern constitution, at least to the extent that condition is indicated by body mass. The relative extent of iridescent coverage was, however, positively related to developmental rate in males ($r = 0.286$, $N = 70$, $P = 0.016$) but not females ($r = 0.060$, $N = 86$, $P = 0.583$). Males that developed faster exhibited phenotypes more greatly dominated by iridescence (at the expense of pigmentary orange).

SEXUAL DIMORPHISM

We assessed sexual differences in colour and life history variables by testing the fixed effect of sex in each single trait GLMM. As noted, variation in overall body size was controlled for relative iridescent coverage in the model by including dorsal body area as a covariate (which, due to the expected relationship between dorsal area and iridescent area, proved highly significant: $F_{1,116.3} = 60.1$, $P < 0.001$; see Fig. 2). The effect of sex was significant in this model ($F_{1,25.2} = 163.4$, $P < 0.001$) as it was in the model conducted on body size itself ($F_{1,137.1} = 248.4$, $P < 0.001$) but not in the model of development time ($F_{1,131.7} = 0.119$, $P = 0.730$). The sexes therefore developed at the same rate (Fig. 3b); however, males matured at a smaller average size than females (GLMM estimate = -44.7 ± 2.84 mm²; Fig. 3a) and exhibited more extensive iridescence (estimate = 62.0 ± 4.85 mm²; Fig. 3c).

RANDOM EFFECTS MODELLING AND GENETIC PARAMETERS

The most parsimonious (co)variance structures for relative iridescent coverage, body size and
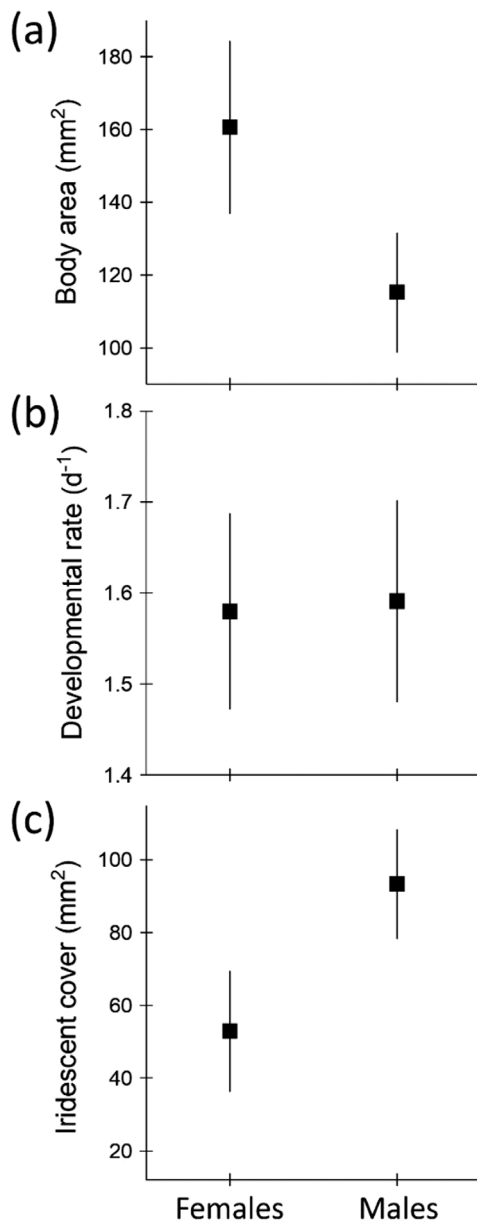
Figure 3. Sexual variation in the three dependent variables of interest in this study. (a) total body area, (b) developmental rate and (c) iridescent cover. Iridescent cover (c) indicates the absolute areal coverage of iridescent markings, and hence is expressed on the same scale as body area (a). Means are shown ± 1 standard deviation. Refer to text for details regarding the relevant statistical contrasts.

developmental rate were defined by the progressive model-fitting procedure for each trait (summarized in Table 1), which produced estimates for random effects as shown in Table 2. These analyses supported significant heritability in all three traits, with estimates nearing 1.0 for development rate. The intersexual genetic correlation was bounded at 1.0 for

both life history traits (which arises because estimates cannot exceed the definite parameter space, generally signifying a true value very close to 1.0). Interestingly, the intersexual genetic correlation for iridescent coverage proved to be significantly negative (i.e. -0.731; Table 2; Fig. 4a). Hence, families in which sons have high iridescence tend to have daughters with relatively low iridescence and vice versa. This result is equivalent to a sex × genotype interaction in which the degree of phenotypic divergence between males and females varies according to genotype (Fig. 4b). The estimable magnitude of both genetic and residual variance for this trait also differed between the sexes, with lower values for males in each case.

MULTIVARIATE GENETIC ARCHITECTURE

The multi-trait model yielded sex-specific estimates for genetic and phenotypic (co)variances among the three studied traits (Table 3). Here we analysed absolute rather than size-corrected iridescent coverage, which we have earlier shown to co-vary phenotypically with body area (Fig. 2). Interestingly, this analysis revealed extremely strong genetic covariance between male iridescent coverage and body area, with the same also true for developmental rate. Genetic correlations among all three traits in males were in fact estimably > 0.76 and not statistically distinguishable from 1.0. Evidence for an equivalent situation in females was less clear because their genetic correlations proved to be more moderate and were accompanied by considerably larger standard errors (Table 3). There was nevertheless a similarly high genetic link between body area and development time in this sex. Phenotypic correlations were overall much lower than genetic correlations, albeit in agreement with the relationships thus demonstrated for iridescent coverage and body area in both sexes (Fig. 2) as well as developmental rate in males.

This model generated broad-sense heritability estimates for absolute iridescent coverage of $H^2 = 0.600 \pm 0.153$ (males) and $H^2 = 0.453 \pm 0.176$ (females). These values are marginally higher those reported earlier for relative coverage (Table 2) because absolute coverage includes a component of genetic variation due to its covariance with body area per se. Multi-trait model estimates for body area ($H^2 = 0.661 \pm 0.130$ & $H^2 = 0.357 \pm 0.193$) were slightly lower than those gained under the single trait model—yet still within the bounds of mutual estimation error—whereas those for developmental rate ($H^2 = 0.954 \pm 0.020$ & $H^2 = 0.931 \pm 0.030$) were near-identical.

DISCUSSION

Our results reveal genetic variation for aposematic coloration in the hibiscus harlequin bug that is

Table 1. Summary of the progressive model-fitting procedure for each single trait GLMM. Starting from a fully constrained model, each step introduced a change to one feature of the variance structure. $P < 0.05$ indicates a significant increase of overall model fit due to this change; these changes were incorporated prior to progressing to the next step. The most parsimonious models indicated in the final row were used to estimate the values of genetic (co)variances and heritability shown in Table 2

		Trait		
Step	Model	Iridescent cover	Body size (area)	Development rate
0	Fully constrained model $(y = sex + G_{mf} + R_{mf})$	LL = -496.4	LL = -88.8	LL = -365.1
1	Residual variances freed across sexes $(R_m \neq R_f)$	LL = -493.1 $G_1 = 6.64, P < 0.05$	LL = -84.1 $G_1 = 9.38, P < 0.005$	LL = -361.2 $G_1 = 7.69, P < 0.01$
2	Genetic variances freed across sexes $(G_m \neq G_f)$	LL = -490.3 $G_1 = 5.53, P < 0.05$	LL = -83.9 $G_1 = 0.334, P = 0.558$	LL = -360.2 $G_1 = 2.04, P = 0.153$
3	Intersexual genetic correlation freed from 1.0 $(G_{m \times f} \neq 1.0)$	LL = -487.4 $G_1 = 5.72, P < 0.05$	LL = -84.1 $G_1 = 0.001, P = 0.998$	LL = -361.0 $G_1 = 0.454, P = 0.816$
4	Plus random "cup" variable $(+G_{cup})$	LL = -487.3 $G_1 = 0.340, P = 0.560$	LL = -84.1 $G_1 = 0.00, P = 0.999$	LL = -335.2 $G_1 = 51.6, P < 0.001$
	Most parsimonious (final) model	$y = sex + G_m + G_f + G_{m \times f} + R_m + R_f$	$y = sex + G_{mf} + R_m + R_f$	$y = sex + G_{cup} + G_{mf} + R_m + R_f$

Abbreviation: LL, Log-Likelihood.

Table 2. Random variance estimates and genetic parameters as obtained from the most-parsimonious single trait GLMMs. Sample sizes: females $N = 86$, males $N = 70$. Genetic parameters significant at $P < 0.05$ are given in bold type. *Parameter bounded to 1.0 (see text)

| Trait	Sex	Genetic variance (σ^2_G)	Residual variance (σ^2_R)	Heritability (H^2)	Intersexual correlation (r_{mf})
Relative iridescent cover	M	90.73	108.0	**0.457 ± 0.180**	**-0.731 ± 0.338**
	F	145.0	220.0	**0.397 ± 0.164**	
Body size (area)	M	362.2	145.4	**0.714 ± 0.103**	1.0 ± 0.0*
	F		431.4	**0.456 ± 0.122**	
Development rate	M	190.4	11.08	**0.945 ± 0.025**	1.0 ± 0.0*
	F		16.10	**0.922 ± 0.033**	

integrated with a tightly constrained genetic architecture for juvenile life history. There were significant genetic variances and, in-turn, high heritability estimates for the proportion of iridescent coloration in both sexes. Body size and development time were also highly heritable, and the three traits were linked by strong genetic correlations, particularly in males. We found little evidence that iridescent coverage is condition-dependent in either sex, at least via the conventional metric of covariance with residual mass. In males there was, however, a positive relationship between development time and iridescent coverage.

In keeping with previous studies of this species (Ballard & Holdaway, 1926; Fabricant et al., 2018;

Medina et al., 2020), we found considerable sexual dimorphism with regards to both size and colour. Males were significantly smaller than females (Supporting Information, Fig. S1) (despite developing at the same rate) and had a higher proportion of their dorsal surface covered by iridescent coloration (although there was considerable overlap, see Fig. 2). Our rearing also revealed a slight female-biased sex ratio, which is consistent with previous laboratory-reared samples of this species (Ballard & Holdaway, 1926).

Given that we found significant heritability for iridescent cover it remains unclear why stabilizing selection by predators has not acted to reduce the considerable colour variation seen in this species.
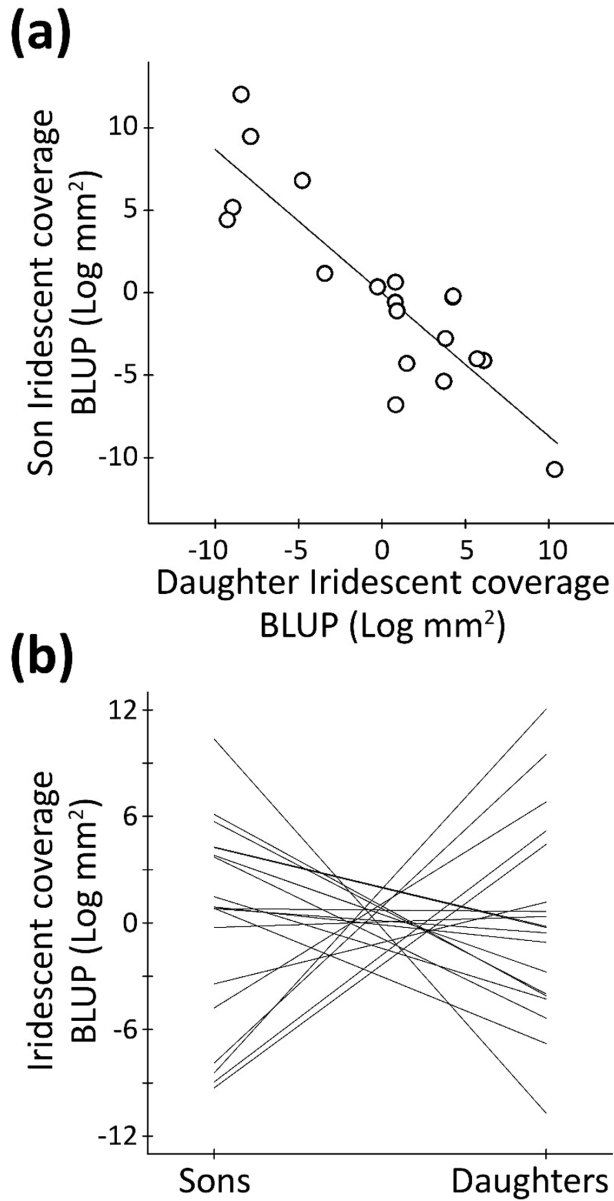
Figure 4. Evidence for the significant sex-by-genotype interaction for iridescent coverage. a, this effect as indicated by the negative intersexual genetic correlation. Each point represents a different putative full-sibling family. Both axes indicate Best Linear Unbiased Predictor (BLUP) values, which are estimates for genetic breeding value obtained from linear mixed modelling. b, the effect as indicated by the variability in sexual reaction norm for iridescent coverage across the sample of full-sibling families. The BLUPs represent size-corrected coverage of dorsal iridescence in all cases.

Possible mechanisms maintaining such variation can be broken down into four main categories: genetic architecture, environmentally-induced

Table 3. Matrices of phenotypic and genetic variances (shaded diagonal), phenotypic correlations (upper off-diagonals) and genetic correlations (lower off-diagonals) obtained from the multi-trait GLMM for each sex. The absolute coverage of iridescence was analysed here, as opposed to size-relative coverage as reported in Table 2. Correlation estimates are given ± 1 standard error. Estimates that are greater than 0.0 and do not significantly differ from 1.0 are indicated in bold, and those that lie statistically between 0.0 and 1.0 are shown with an asterisk. Others are either not significantly different from 0.0 or cannot be distinguished based upon their standard error. Heritability estimates derived from the variance estimates are reported in the text

	Males			Females		
	Iridescent cover	Body size (area)	Development rate	Iridescent cover	Body size (area)	Developmental rate
Absolute iridescent cover	$\sigma_P^2 = 386.3$ $\sigma_G^2 = 231.8$	$0.663^* \pm 0.081$	$0.300^* \pm 0.137$	$\sigma_P^2 = 406.6$ $\sigma_G^2 = 184.0$	$0.350^* \pm 0.109$	-0.019 ± 0.130
Body size (area)	$\mathbf{0.901 \pm 0.110}$	$\sigma_P^2 = 440.5$ $\sigma_G^2 = 291.0$	0.100 ± 0.151	0.608 ± 0.347	$\sigma_P^2 = 709.1$ $\sigma_G^2 = 253.2$	-0.157 ± 0.131
Developmental rate	$\mathbf{0.854 \pm 0.146}$	$\mathbf{0.763 \pm 0.168}$	$\sigma_P^2 = 258.2$ $\sigma_G^2 = 246.3$	0.356 ± 0.336	$\mathbf{0.735 \pm 0.268}$	$\sigma_P^2 = 232.1$ $\sigma_G^2 = 216.0$

variation, relaxed selection and sexually-antagonistic selection.

The high genetic correlation between coloration, development time and body size indicate that the genes underlying colour formation are also involved in, or closely linked with, development and growth. This was particularly the case in males, and could reflect either pleiotropy or genetic linkage. Thus selection acting on development may also impact coloration, potentially dragging it off its adaptive optima. If selection on development varies between populations, this may then create the coloration differences we see in nature. However, in our experiment we kept all animals in standardized laboratory conditions, without the environmental fluctuations that often occur in their natural environment. Thus, while our findings suggest a fairly tightly controlled genetic architecture, this may give an unrealistic view of developmental outcomes in the field. Nevertheless, hibiscus harlequin bugs do spend the majority of their nymphal instars living in sibling groups (E. Burdfield-Steel, pers. obs.), meaning related individuals should experience similar conditions [although non-kin can also aggregate, see Jones (2020)]. This group living may be connected to the very high heritability seen in developmental time and size, in order to synchronize both moulting time and size at moult. This could help to increase signal uniformity within the groups, perhaps presenting a stronger anti-predator signal (Sillén-Tullberg, 1990), or reducing the risk of sibling cannibalism (Mukai *et al.*, 2018). Previous work has demonstrated the role of temperature plasticity in adult coloration in this species (Fabricant *et al.*, 2018), although it is also not yet clear if this plasticity is adaptive, or simply a side effect of the difficulties in laying down the melanization layers needed to produce the iridescence at higher temperatures (Gibert *et al.*, 2007; Fabricant *et al.*, 2013). If coloration plays a large role in thermoregulation the ability to plastically respond to the environment during development may be of greater importance than possessing an optimal anti-predator signal during adulthood. Although the role of melanization in thermoregulation is well-supported (Solensky & Larkin, 2003), existing evidence does not support the idea that iridescence coloration can play a similar role (Schultz & Hadley, 1987; Doucet & Meadows, 2009), although this has not yet been tested in this species. Evidence that plasticity in coloration may be adaptive comes from the findings of Fabricant *et al.* (2018) that this plasticity varies between populations. In addition to temperature, it has been suggested that variation in predator community may be driving many of the population-level differences seen. In particular, mantids, which are undeterred by the bugs' chemical defences (Fabricant & Smith, 2014) and select for more orange bugs due to their visual system (Fabricant & Herberstein, 2014), may be more common in tropical regions and when temperatures are higher (S. Fabricant, unpublished data). Thus temperature plasticity may also allow this species to adapt to the local and temporal predator community. This is notable as while the bugs are more numerous during the summer months (D. J. Kemp, pers. obs.), they can have several generations per year and thus different individuals can experience very different conditions. A similar pattern was found in the poison frog, *Oophaga granulifera*, where differing predation pressures from birds and lizards have been suggested as a driver of geographic variation in colour (Willink *et al.*, 2014).

However, it is not yet clear just how strong selection by predators actually is in the wild. Although previous studies have suggested that the combination of orange and blue markings presents the strongest signal to birds (Fabricant *et al.*, 2014), given the distinctiveness of the orange and blue coloration it may be that predators are able to easily generalize between the bugs, reducing selection for signal conformity (Medina *et al.*, 2020). Predator generalization between distinct colour morphs is known to occur in other iridescent insects (Kikuchi *et al.*, 2020), and it has previously been found that wild-caught great tits were able to generalize between iridescent bugs and those with their iridescent patches painted black, which may also allow the hibiscus harlequin bug to benefit from the presence of aposematic black and red bugs in their environment, as well as conspecifics (Fabricant & Herberstein, 2014; Fabricant & Smith, 2014). There is also evidence that predators generalize between adults and nymphs (Medina *et al.*, 2020). The nymphs of this species often co-occur with adults on host plants and show high levels of iridescent coverage at the later stages of development. When comparing the level of chemical defence across sexes and life-stages, Medina *et al.* (2020) found that males showed the highest levels of toxicity relative to body weight, followed by final-instar nymphs and then adult females (Medina *et al.*, 2020); however, it is not yet clear if predators differentiate between the groups based on this.

Another trait that differs between different populations of the hibiscus harlequin bug is sexual dimorphism. Of all the populations sampled by Fabricant *et al.* (2018) the Sydney population, the focus of our study, showed the lowest sexual dimorphism. Nevertheless we found a negative relationship between the level of iridescence seen in brothers and sisters: bluer brothers had more orange sisters. Thus, some families showed more marked sexual dimorphism than others. Explaining this pattern is a challenge as we do not know what the optimum colour pattern

is for either sex, or how changes in environment and predator community may alter this. One possibility is that, given that the extent of sexual dimorphism varies geographically, there is gene flow such that dimorphic genotypes intermix with more monomorphic genotypes from elsewhere. In tropical populations the bugs almost appear to have distinct morphs, as wild males show either very high levels of iridescence or none at all, while all females lacked iridescence patches entirely. In contrast, the temperate population studied here shows more continuous colour variation in both sexes, although mean male iridescent coverage remains higher than that of females. If these patterns are the result of differing selection pressures, be they natural or sexual, limited gene flow between the populations could maintain variation (Gordon *et al.*, 2015). However, this pattern is further complicated by the temperature sensitive plasticity in coloration. Although the dimorphism seen in males from the tropical populations persisted in the laboratory, even under lower rearing temperatures, tropical females reared at lower temperatures did show intermediate levels of iridescent coverage (Fabricant *et al.*, 2018). It may well be that the temperature sensitivity of these bugs in terms of the development of iridescence varies both between populations and sexes, producing the different patterns observed. Differing optima between males and females may also explain why the genetic correlation across life-history traits is less tight in females compared to males. Finally, while iridescence can act as a sexual signal in groups such as birds (White, 2020) and butterflies (Kemp, 2007), and a role of sexual selection in producing colour dimorphism in hibiscus harlequin bugs cannot be ruled out (Keller, 2012), it does seem unlikely as colour-based mate choice has never been described in scutellerid bugs. Instead it is likely that chemical (Aldrich, 1995) or mechanical (Čokl, 2008) signals form the basis for sexual communication in this species.

CONCLUSION

The negative genetic correlation found between iridescent coverage in males and females suggests that there may be divergent selection for coloration acting on the two sexes in the hibiscus harlequin bug. Why some families show a higher degree of sexual dimorphism than others though remains unclear. Nevertheless, as coloration is significantly heritable in both sexes, there must either be relaxed selection for coloration acting in this species, or some other process is maintaining the high levels of variation seen in the wild. Different optima for coloration in males and females may be contributing to this maintenance,

particularly if sexual dimorphism is in the process of evolving in this species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Allometric relationships between body length and fresh body weight. Relationships are described by the fitted power curves $y = 0.014x^{3.425}$ (males) and $y = 1.93x^{1.663}$ (females).

SHARED DATA

The data underlying the study have been deposited in the Dryad digital repository (Burdfield-Steel & Kemp, 2021).