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Morph-specific variation in intersexual genetic correlations in an intra-specific mimicry system

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ABSTRACT

Background: Positive intersexual genetic correlations are typically viewed as constraining the evolution of sexual dimorphism, when traits are subject to sexually antagonistic selection. Our study species, the damselfly *Ischnura elegans*, has a female-limited colour polymorphism with three female colour morphs (males are monomorphic), one of which is considered to be a male mimic.

Questions: Are there morph-specific differences in the magnitude of intersexual genetic correlations in *I. elegans*? Specifically, do male-mimic (Androchrome) females have higher intersexual genetic correlations for morphological traits than non-mimic (Infuscans) females?

Methods: We collected copulating pairs in the field and raised offspring from these pairs in the laboratory. We measured five morphological traits in both parent and offspring generations and investigated their heritabilities and genetic correlations.

Results: We found a negative overall relationship between the degree of sexual dimorphism for a trait and its intersexual genetic correlation. But the magnitude and direction of intersexual genetic correlations depended on the female morph. As expected, male mimic (Androchrome) females had higher intersexual genetic correlations. In addition, the genetic correlations between the morphs were in all cases significantly lower than unity. Male mimic (Androchrome) females had higher mother–son covariances than the non-mimic (Infuscans) morph, and this difference is the proximate explanation for the difference in intersexual genetic correlations between the morphs.

Keywords: damselflies, *Ischnura elegans*, male mimic, polymorphism, sexual dimorphism.

INTRODUCTION

Sexual dimorphism, the existence of consistent morphological differences between males and females, is a common feature of many sexually reproducing organisms (Andersson, 1994). However, theory predicts that phenotypic traits should by default start off as being highly correlated between the sexes (Lande, 1980). As sexual dimorphism evolves, we might therefore expect that these intersexual genetic correlations have subsequently been broken down over time (Bonduriansky and Chenoweth, 2009). A few studies have indeed found low intersexual genetic

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correlations that are significantly different from unity (Chippindale *et al.*, 2001; Bonduriansky and Rowe, 2005; Harano and Miyatake, 2007; Steven *et al.*, 2007; Poissant *et al.*, 2008). However, many other studies have found high genetic correlations between the sexes, even in sexually dimorphic traits (Merilä *et al.*, 1998; Jensen *et al.*, 2003; Long and Rice, 2007; Sakai *et al.*, 2008). This suggests that constraints in the magnitude and/or speed of the evolution of sexual dimorphism may be common. If high intersexual genetic correlations are combined with sexually antagonistic selection on the same traits, this results in intra-locus sexual conflict (Chippindale *et al.*, 2001; Rice and Chippindale, 2001). There is evidence that sexually antagonistic selection pressures are common in natural populations (Cox and Calsbeek, 2009), and that intra-locus sexual conflict can lead to substantial evolutionary costs and a significant gender load for fitness (Pischedda and Chippindale, 2006; Prasad *et al.*, 2007). We might therefore expect that selection should act to reduce intersexual genetic correlations when males and females have different optimal trait values.

Positive intersexual genetic correlations are therefore typically viewed as constraining the evolution of sexual dimorphism. However, genetic correlations need not always be maladaptive and constrain adaptive evolution. They could also be adaptive, and result from selection for optimal character combinations (Sinervo and Svensson, 2002). When correlational selection favours optimal combinations of different traits, one expected outcome may be the build-up and maintenance of adaptive genetic correlations and genetic integration between suites of traits (Pigliucci and Preston, 2004). The dual nature of genetic correlations (as either constraints or an adaptive outcome of selection) is important to keep in mind, since both views are logically justified, and their relative importance is a key empirical issue.

Here, we investigate and discuss the intersexual genetic correlations in a polymorphic damselfly species, *Ischnura elegans*. This species has female-limited polymorphism (i.e. females are polymorphic but males are monomorphic) and has proved to be an interesting study organism for a range of different questions in evolutionary biology (Svensson *et al.*, 2005; Gosden and Svensson, 2007; Abbott and Svensson, 2008). One of the female morphs in this species has similar coloration to males and is considered to be a male mimic (e.g. Cordero *et al.*, 1998). Recent evidence also suggests that Androchromes may have male-like morphology in some species (Van Gossum *et al.*, 2008; Abbott and Gosden, 2009). We therefore expected that patterns of intersexual genetic correlations for morphological traits could be dependent on female morph. Specifically, we expected that Androchromes would show more positive correlations between the sexes than the other morphs. The exact mechanism(s) resulting in Androchromes' morphological similarity to males is unknown, but could be due to correlational selection for more effective male mimicry, a correlated response to selection on morphology resulting from alternative adaptive strategies associated with each morph, or simply pleiotropic effects of the morph locus. In this paper, we present data on the magnitude of intersexual genetic correlations [i.e. $r_{\mathbf{MF}}$ (Bonduriansky and Rowe, 2005)], and on morph-specific intersexual genetic correlations ($r_{\mathbf{MA}}$, the genetic correlation between males and the Androchrome morph and $r_{\mathbf{MI}}$, the genetic correlation between males and the Infuscans morph) and inter-morph genetic correlations ($r_{\mathbf{AI}}$, the genetic correlation between the Androchrome and Infuscans morphs).

We found a negative overall relationship between the degree of sexual dimorphism for a trait and its intersexual genetic correlation, but that the magnitude and direction of intersexual genetic correlations was indeed dependent on female morph, suggesting incomplete sex-limitation of morph locus effects. In this particular polymorphic system, high intersexual genetic correlations may actually be part of an adaptive response to selection for male mimicry via correlational selection. These results contrast with the

standard view of intersexual genetic correlations as an evolutionary constraint (Merilä *et al.*, 1998; Jensen *et al.*, 2003; Long and Rice, 2007; Sakai *et al.*, 2008). In addition, the genetic correlations between the morphs were in all cases significantly lower than unity. Low between-morph genetic correlations would facilitate morphological divergence of these morphs, in the same way as low intersexual genetic correlations would facilitate the evolution of sexual dimorphism. Despite the obvious relevance of these results to this and other species with female-limited colour polymorphism, which is common in damselflies (Corbet, 1999), the unique nature of this non-model system and the novel results obtained from it will hopefully be a useful general addition to our knowledge of the evolution of sexual dimorphism.

METHODS

Study species

Ischnura elegans is a small European damselfly with a north–south distribution ranging from southern Sweden to northern Spain (Askew, 1988). As is relatively common in the *Ischnura* genus, *I. elegans* has a female-limited colour polymorphism and females may belong to one of three different morphs: Androchrome, Infuscans or Infuscans-obsoleta. Males are monomorphic and hence this colour polymorphism is sex-limited in its expression (Cordero, 1990; Sánchez-Guillén *et al.*, 2005). Infuscans and Infuscans-obsoleta have more cryptic black and olive green or brown coloration when mature, while Androchrome females, which are considered to be male mimics, have blue and black coloration similar to males (Askew, 1988). [For colour pictures of the morphs and their developmental colour stages, see Svensson *et al.* (2009a).] Female colour morph is determined by one locus with three alleles in a dominance hierarchy, where the Androchrome allele is dominant to both other alleles and the Infuscans-obsoleta allele is recessive to both other alleles, with the Infuscans allele recessive to the Androchrome allele and dominant to the Infuscans-obsoleta allele (i.e. A > I > O, where A = Androchrome allele, I = Infuscans allele, and O = Infuscans-obsoleta allele) (Sánchez-Guillén *et al.*, 2005).

Data collection

Individuals used in this study were collected in 2002 from Vombs Vattenverk, one of our study populations outside of Lund [see Abbott *et al.* (2008) for the location of this population]. We captured wild individuals of *I. elegans* found in copula, and here we assume that the male that was captured in copula with the female actually sired most of her offspring. This is reasonable since *I. elegans* has last male precedence (Cooper *et al.*, 1996) and, in support of this assumption, we found that maternal and paternal heritabilities were similar for most traits (data not shown). This result is consistent with the assumption that the male captured in copula did in fact fertilize the majority of the eggs, and that incorrectly assigned paternity is therefore not a major problem in this study. We took five morphological measurements from both sexes in each copulating pair we caught in the field: total body length, abdomen length, thorax width, width of the fourth segment of the abdomen, and forewing length. These morphological measures were selected for analysis since they are easy to measure, have been used in previous analyses of larval morphology and adult morphology (Abbott and Svensson, 2008; Abbott and Gosden, 2009), and are known to be sexually dimorphic (Abbott and Svensson, 2008; Abbott and Gosden, 2009). Each measurement was taken a

minimum of twice, to the nearest 0.01 mm. Repeatabilities for morphological measurements were all >90% (Lessells and Boag, 1987). After being measured, the male was released and the female taken to the laboratory for oviposition. Females oviposited onto damp filter paper in small plastic cups for 48 h, and were then released. The eggs obtained in this way were stored in water in the small plastic cups until hatching. Once hatched, larvae were transferred to larger containers and fed with brine shrimp (*Artermia* sp.) daily. After approximately 1 month, the larvae were moved to individual enclosures within the large containers to prevent cannibalism. Because of time and space constraints, no more than 20 individuals per family could be placed in individual enclosures. Once larvae had undergone metamorphosis and emerged as adults the following spring, the same five morphological measurements were taken as in the parental generation, and sex and morph were recorded.

Data analysis

Because males and females differ in size, morphological measures were corrected for sex before calculating heritabilities and genetic correlations. Correction was done separately for parent and offspring generations because they differed in overall size (this is probably due to environmental factors, since parents were wild-caught and offspring were raised in the laboratory). To correct for sex, first the difference in mean size between the sexes was calculated. The mean difference was then multiplied by 0.5 and either added (in the case of the smaller sex) or subtracted (in the case of the larger sex) to each measurement. This eliminated mean size differences between the sexes. Narrow-sense heritabilities and genetic correlations between traits were then calculated from mid-parent–offspring regressions weighted by offspring number using the software H2boot (Phillips, 2001). This program generates an error distribution for each parameter using bootstrapping methods and calculates significance of estimates from this distribution. In total, 59 families were included in this analysis (mean offspring number per family ± S.D.: 7.58 ± 2.29, min = 3, max = 12). Houle's evolvability (I_A) and the coefficient of additive genetic variance (CV_A) were also calculated for each trait (Houle, 1992). Full-sib data from these same 59 families were used to estimate heritability of development time using a weighted analysis (note that it was not possible to use parent–offspring data for this analysis since the development time of the parents was unknown).

To estimate intersexual genetic correlations (r_{MF}), a similar analysis to that for between-trait genetic correlations (see above) was carried out using uncorrected trait values. Intersexual genetic correlations were calculated as the mean cross-sex covariance divided by the product of the within-sex covariances (Falconer and Mackay, 1996). Families with adult offspring of only one sex were excluded from this analysis, which therefore included a total of 48 families. Data were weighted by offspring number of each sex and parameters were tested for significance using bootstrapping (mean female offspring per family ± S.D.: 3.04 ± 1.58, min = 1, max = 7; mean male offspring per family ± S.D.: 4.58 ± 2.01, min = 1, max = 10). Following work by Bonduriansky and Rowe (2005), we also calculated the correlation between the degree of sexual dimorphism in a trait and the intersexual genetic correlation for that trait to test the existence of a negative relationship between the two. Degree of sexual dimorphism was estimated as the difference in mean trait size between the sexes expressed as a percentage of the mean for all individuals, i.e. (female mean – male mean)*100/grand mean.

To determine if the magnitude and direction of intersexual genetic correlations was dependent on female morph, separate analyses were carried out on Androchrome families and Infuscans families. Families were classified by maternal morph in this analysis, so data for Androchrome families, for example, comprised the Androchrome mother, her mate, all her male offspring, and her Androchrome offspring. All female offspring that were of a different morph than their mother were therefore excluded from this analysis, which lowered the sample size for the rarest morph (Infuscans-obsoleta). Because the Infuscans-obsoleta allele is recessive to both other alleles, very few Infuscans-obsoleta females produced Infuscans-obsoleta offspring, so unfortunately this morph could not be analysed separately for intersexual genetic correlations. Sample sizes for Androchrome families and Infuscans families were 23 and 12 respectively, and analyses were weighted by offspring number of each sex (for Androchromes: mean female offspring per family ± S.D.: 2.22 ± 1.13, min = 1, max = 5 and mean male offspring per family ± S.D.: 4.26 ± 1.76, min = 1, max = 8; for Infuscans: mean female offspring per family ± S.D.: 1.33 ± 0.65, min = 1, max = 3 and mean male offspring per family ± S.D.: 4.17 ± 2.25, min = 2, max = 10). We investigated if there were significant differences in the magnitude of intersexual correlations between these two morphs using a bootstrapping procedure in the software Resampling Stats (Simon, 2000). We obtained *P*-values by generating an error distribution for each morph-specific intersexual genetic correlation and then testing this distribution for overlap with the corresponding estimate in the other morph. Since there was evidence that intersexual genetic correlations were higher in Androchrome females, we also calculated degree of sexual dimorphism separately for each morph and tested for a difference using a paired *t*-test. In addition, we carried out a factorial analysis of variance (ANOVA) of parent–offspring covariances to determine which relationships were driving differences in the magnitude of r_{MF} between morphs. For this analysis, the four covariances used in calculating intersexual genetic correlations (mother–son, father–daughter, mother–daughter, and father–son) acted as the dependent variable, and were calculated separately by morph for each trait, for a total sample size of 40 (i.e. four covariances × five traits × two morphs = 40). The covariances were then analysed in a model with Type of covariance (i.e. mother–son, father–daughter, etc.), Trait (to control for differences in covariances due to trait size), and Morph as factors, and all two-way interactions included. A significant interaction between Type and Morph means that different types of covariance vary in magnitude between morphs, and may indicate what is causing variation in the magnitude of r_{MF} between morphs. The factorial ANOVA and paired *t*-tests discussed above were carried out in STATISTICA (Statsoft, Inc. 2004).

Genetic correlations between two of the female morphs (Androchrome and Infuscans) were also investigated. As with the analysis of morph-specific intersexual genetic correlations, the rarest morph, Infuscans-obsoleta, had to be excluded due to low sample size. Both types of parent–offspring 'cross-morph' covariances (i.e. the covariance between Androchrome mothers and Infuscans daughters, and the covariance between Infuscans mothers and Androchrome daughters) cannot be calculated using the same set of families, since mothers are either phenotypically Androchrome or Infuscans (not both), and there is currently no marker available for the morph locus in this species. Thus for this analysis, it was necessary to use full-sib data instead of parent–offspring data. Inter-morph genetic correlations (the genetic correlation between Androchrome and Infuscans females, r_{AI}) were calculated from within-family morph means as the covariance between full-sib sisters of each morph divided by the product of the standard deviations for each morph (Falconer and

Mackay, 1996), and estimated from 24 families of full-sib sisters containing different morphs. Data were weighted by offspring number (mean sisters per family ± S.E.: 3.79 ± 1.56, min = 2, max = 7). This analysis was carried out in Resampling Stats (Simon, 2000), but used a similar method as H2boot (i.e. generating an error distribution for each parameter using bootstrapping methods and calculating significance of estimates from this distribution).

Caveats

The two methods for estimating quantitative genetic parameters used here each have limitations. Parent–offspring analysis using wild-caught parents and laboratory-raised offspring can potentially confound environmental and genetic effects, while full-sib analysis potentially confounds maternal and genetic effects (Falconer and Mackay, 1996). The ideal data set for this sort of analysis would have been obtained from a multi-generational half-sib experimental design with all individuals raised in a common environment (Falconer and Mackay, 1996). However, due to time and space constraints, this was not possible. Raising one generation of *I. elegans* in the laboratory under constant conditions takes 6–10 months (Abbott and Svensson, 2005) and does not result in synchronized emergence of adult damselflies similar to that seen in natural populations (J.K. Abbott, personal observation). These factors make implementing planned crosses over several generations laborious, although of course not impossible (e.g. Sánchez-Guillén *et al.*, 2005). With limited space, there is also a trade-off between the number of populations that can be investigated and the accuracy of estimates for each population (i.e. number of individuals per population that can be obtained), so we elected to increase the number of individuals from a single target population (Vombs Vattenverk) rather than try to investigate several populations simultaneously but with very low power. Although the data presented here are limited, we feel that these results still have value due to their novelty and source from a non-model organism. In addition, previous work has shown that heritabilities and genetic correlations obtained from wild-caught parents and laboratiry-raised offspring can provide a lower bound for actual heritabilities and genetic correlations (Riska *et al.*, 1989). We also attempted to check reliability of parent–offspring estimates of intersexual genetic correlations by calculating the same parameters from full-sib data, and testing for a positive correlation between the two. Intersexual genetic correlations estimated from parent–offspring and full-sib data sets were indeed significantly correlated ($r = 0.56$, $P = 0.038$), although full-sib estimates were lower and had a more narrow range (between 0.04 and 0.72) than the parent–offspring estimates. Nevertheless, this suggests that the relative magnitudes of the intersexual genetic correlations estimated from parent–offspring data are reasonably accurate, so only the parent–offspring estimates are presented here.

RESULTS

Of the five morphological traits investigated in this study, only thorax width and width of the fourth segment of the abdomen (S4) lacked significant heritable variation (Table 1). Low repeatability of measurements is unlikely to account for the lack of significant heritability for these traits since all traits had repeatabilities >90% (see Methods). However, lack of significant heritability for thorax width may instead be due to low statistical power (i.e. low number of families), since all the genetic correlations between traits were significantly

Table 1. Heritabilities, genetic correlations, and genetic covariances for five morphological traits

	Total length	Abdomen length	Thorax width	S4 width	Wing length
Total length	**0.299 (0.096)**	**1.117 (0.426)**	**1.190 (0.664)**	0.566 (1.212)	**0.797 (0.262)**
Abdomen length	**0.272 (0.125)**	**0.194 (0.107)**	**1.001 (0.638)**	0.438 (1.295)	**0.737 (0.340)**
Thorax width	0.016 (0.008)	0.009 (0.007)	0.092 (0.102)	0.932 (1.731)	**1.133 (0.681)**
S4 width	0.006 (0.005)	0.003 (0.005)	0.0003 (0.0004)	0.065 (0.061)	0.916 (1.493)
Wing length	**0.181 (0.083)**	**0.111 (0.061)**	**0.009 (0.005)**	0.006 (0.004)	**0.537 (0.222)**

Note: Genetic correlations are located above the diagonal, heritabilities along the diagonal, and genetic covariances below the diagonal. Standard deviations are reported in parentheses. Genetic correlation estimates that are significantly different from zero are highlighted. Genetic correlations reported >1 are not significantly different from +1.

Table 2. Quantitative genetic parameters for five morphological traits

Trait	V_P	V_A	I_A	CV_A	P-value
Total length	1.268 (0.302)	0.388 (0.160)	$3.25*10^{-4}$	1.804	**0.007**
Abdomen length	0.854 (0.199)	0.173 (0.104)	$2.27*10^{-4}$	1.507	**0.047**
Thorax width	0.007 (0.001)	0.0006 (0.0006)	$9.39*10^{-5}$	0.969	0.192
S4 width	0.006 (0.001)	0.0004 (0.0004)	$6.40*10^{-4}$	2.530	0.155
Wing length	0.264 (0.042)	0.141 (0.061)	$3.57*10^{-4}$	1.891	**0.003**

Note: V_P = phenotypic variance, V_A = additive genetic variance, I_A = Houle's evolvability, CV_A = coefficient of additive genetic variance. P-value refers to the probability that $V_A = 0$, with significant values highlighted. Standard deviations are reported in parentheses. All traits have significant additive genetic variance except thorax width and abdomen width (S4), although S4 width has the highest evolvability and CV_A.

different from zero, except those involving S4 (Table 1). Interestingly, S4 had the highest evolvability and highest coefficient of additive genetic variation, despite the low heritability for this trait (Table 2). The heritability value for development time was also significantly greater than zero ($h^2 = 0.515 \pm 0.105$, $P < 0.001$), although strictly speaking we cannot infer significant heritability of this trait because it is calculated from full-sib data and may be influenced by maternal effects and dominance variance.

The magnitude and direction of intersexual genetic correlations varied between traits, from negative (for S4) to positive correlations not significantly different from +1 (Table 3A). There was a significant negative relationship between the amount of sexual dimorphism in a trait and its intersexual genetic correlation ($r = -0.935$, $P = 0.020$), although the high standard errors for the intersexual genetic correlations and low number of traits included in this analysis means that this relationship cannot be considered conclusive.

The magnitude and sign of the intersexual genetic correlations was dependent on female morph (Table 3B). Two of these intersexual genetic correlations (total length and abdomen length) differed significantly between the morphs, and intersexual genetic correlations for the other three traits also tended towards significance ($P < 0.10$, Table 3B). In four out of five cases, the intersexual genetic correlation between Androchromes and males ($r_{\mathbf{MA}}$) was higher than the intersexual genetic correlation between Infuscans and males ($r_{\mathbf{MI}}$),

Table 3. Intersexual and inter-morph genetic correlations for five morphological traits

(A) Trait	r_{MF}
Total length	**2.230 (1.094)**
Abdomen length	4.087 (2.877)
Thorax width	0.816 (1.557)
S4 width	−0.836 (1.834)
Wing length	**2.328 (1.153)**

(B) Trait	r_{MA}	r_{MI}	*P*-value
Total length	**4.052 (1.911)**	−1.549 (1.729)	**0.004**
Abdomen length	1.265 (0.974)	−1.760 (0.999)*	**0.018**
Thorax width	0.120 (0.852)	1.550 (0.606)	0.053
S4 width	0.174 (1.441)	−0.649 (0.429)*	0.095
Wing length	**1.194 (0.701)**	0.711 (0.524)	0.059

(C) Trait	r_{AI}
Total length	**0.284 (0.132)***
Abdomen length	**0.305 (0.146)***
Thorax width	**0.457 (0.182)***
S4 width	0.063 (0.153)*
Wing length	**0.354 (0.120)***

Note: Intersexual genetic correlations are reported for (A) pooled data for all families with offspring of both sexes, and (B) calculated separately according to female morph. r_{MF} is the overall genetic correlation between males and females; r_{MA} is the genetic correlation between males and Androchrome females; and r_{MI} is the genetic correlation between males and Infuscans females (see text for details). *P*-value refers to the probability that $r_{MA} = r_{MI}$. (C) Inter-morph genetic correlation r_{AI} is the genetic correlation between Androchrome females and Infuscans females. Standard deviations are reported in parentheses. Genetic correlations that are significantly greater than zero are shown in **bold**, and genetic correlations that are significantly smaller than +1 are marked with an asterisk. Although estimates of intersexual genetic correlations may be >1, the true value cannot exceed 1 (see text for details).

the exception being thorax width (Table 3B). This difference between the morphs is qualitatively consistent with the idea that Androchromes are male mimics (mean $r_{MA} = 1.36$, mean $r_{MI} = -0.34$). Indeed, the amount of sexual dimorphism was significantly smaller for Androchrome females than for Infuscans females ($t_4 = -3.17$, $P = 0.03$), consistent with previous findings that Androchromes are morphologically similar to males (Abbott and Svensson, 2008; Abbott and Gosden, 2009). The ANOVA of covariances had only one significant factor, the Type*Morph interaction (Table 4), which suggests that higher intersexual genetic correlations in Androchromes are driven by a high covariance between mothers and sons (Fig. 1).

Table 4. Results of ANOVA of parent–offspring covariances

Effect	d.f.	MS	*F*	*P*-value
Type	3	0.014	0.982	0.433
Trait	4	0.034	2.356	0.112
Morph	1	0.043	2.984	0.110
Type*Trait	12	0.010	0.684	0.740
Type*Morph	**3**	**0.053**	**3.649**	**0.044**
Trait*Morph	4	0.019	1.330	0.314
Error	12	0.014		

Note: Type refers to the type of covariance (mother–son, father–daughter, mother–daughter, or father–son). Trait refers to the morphological trait for which the covariance was calculated and was included to control for differences in covariances due to trait size. Morph refers to whether the covariance was calculated for the Androchrome or Infuscans data set. The significant Type*Morph interaction indicates that different types of covariance vary in magnitude between morphs.

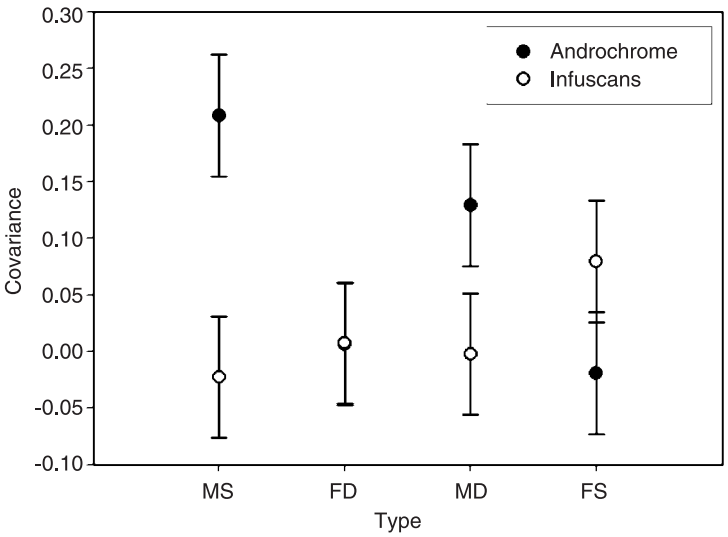

Fig. 1. The magnitude of four different parent–offspring covariances according to female morph. *Abbreviations*: MS = mother–son, FD = father–daughter, MD = mother–daughter, FS = father–son. Androchromes have significantly higher mother–son covariances than Infuscans females (LSD *post hoc* test, $P = 0.01$). Error bars denote standard errors. Note that father–daughter covariances were almost exactly equal for the two morphs, so the symbols overlap in this case.

Finally, inter-morph genetic correlations were all significantly less than +1, and four of five of these genetic correlations differed significantly from zero, the exception being abdomen width (S4; Table 3C). This suggests that high inter-morph genetic correlations are unlikely to constrain the female morphs from developing different morphologies, and females need not necessarily have the same morphology as their sisters of different morphs from the same family.

DISCUSSION

Previous studies on other species have shown that intersexual genetic correlations (r_{MF}) for sexually dimorphic traits may be less than unity (Chippindale *et al.*, 2001; Bonduriansky and Rowe, 2005; Harano and Miyatake, 2007; Steven *et al.*, 2007; Poissant *et al.*, 2008). The results of this study demonstrate morph-specific variation in intersexual genetic correlations. This phenomenon could either be due to increased breakdown of intersexual genetic correlations in the Infuscans morph, or differential build-up of correlations in the male mimic Androchrome morph due to correlational selection. Although the adaptive significance of the female polymorphism in this and other damselfly species has been discussed extensively in the past and subject to much experimental work (Cordero *et al.*, 1998; Abbott and Svensson, 2005, 2008; Svensson and Abbott, 2005; Gosden and Svensson, 2007; Abbott *et al.*, 2008), and the genetics of this polymorphism is well established (Sánchez-Guillén *et al.*, 2005), our knowledge of the developmental and physiological mechanisms behind these morphs is still limited.

Several of the morphological traits we examined here were significantly heritable and most genetic correlations between traits were also significant (Table 1). Since consistent morphological differences between the female morphs have been found across populations of *I. elegans* (Abbott and Gosden, 2009) and females raised in a common laboratory environment differ in morphology according to morph (Abbott and Svensson, 2008), some additive genetic variance for these traits was expected *a priori*. In fact, even the traits with no significant heritability do not appear to be entirely plastic. Thorax width was genetically correlated with total length, abdomen length, and wing length, which suggests that low heritability for this trait may be due to low statistical power. Abdominal width (S4) was not significantly heritable, yet had the highest evolvability and coefficient of additive genetic variance (Houle, 1992) of all five traits (Table 2). This is possible if the amount of phenotypic variation in the trait is large relative to the additive genetic variation, but the additive genetic variation is large relative to the trait mean (Houle, 1992). Similarly, a previous analysis of development time in the laboratory found significant differences in development time between families (Abbott and Svensson, 2005), consistent with the relatively high heritability value we found here for this trait. It is also worth noting that some of the heritabilities and genetic correlations presented here have confidence limits that include zero but are still reported as significant. This is because significance testing for these parameters is carried out on the genetic variance and covariance values and not on the ratios themselves. In these cases, we can therefore conclude that significant additive genetic variance/covariance for these traits does exist, even if the confidence limits for the heritabilities/correlations include zero.

Males and females of *I. elegans* are sexually dimorphic in size and shape (Abbott and Svensson, 2008; Abbott and Gosden, 2009), and we found the predicted negative relationship between the degree of sexual dimorphism for a trait and its intersexual genetic correlation. This result is similar to that of Bonduransky and Rowe (2005) for morphological traits in the fly *Prochyliza xanthostoma*, although the results presented here are unfortunately less robust due to the low number of traits measured. Note that although some of the estimates of r_{MF} presented in Table 3 are substantially higher than 1, the true value of r_{MF} cannot by definition exceed 1. These high estimates are a mathematical result of dividing high cross-sex covariances with low within-sex covariances, and in our view should not be considered biologically realistic, but simply be taken to indicate that intersexual genetic correlations in these cases are high and positive (i.e. probably not significantly different from +1).

There was also evidence of morph-specific differences in the magnitude of intersexual genetic correlations. Although standard deviations for the intersexual genetic correlations are large due to the limited number of families included in each analysis, in all cases the difference between the morphs in r_{MF} was at or near significance (Table 3B). The fact that such a clear pattern of morph-specific intersexual genetic correlations was obtained, despite the low power of this analysis, suggests that r_{MF} is indeed contingent upon female morph. It is also worth noting that this result was obtained using individuals from a single population, and therefore cannot be a spurious pattern resulting from correlated among-population differences in morphology and morph frequencies. These results suggest that it may be useful to take morph identity into account when calculating quantitative genetic parameters in polymorphic species such as *I. elegans*. A limitation to this analysis is of course that the genotype at the morph locus is unknown, although females of a given morph must obviously have at least one copy of the allele for their morph (see 'Study species' above). Males included in the morph-specific r_{MF} analysis may therefore not have had the same morph as their mates and mothers. This will make the estimates of r_{MA} and r_{MI} less precise, and in combination with the low number of families included in the analysis probably explains the large standard errors we obtained. However, given that what is of interest here is the existence of differences between the morphs and not the accuracy of the estimates *per se*, the low power of this analysis only serves to make our calculations more conservative and increase the likelihood that we have detected a true qualitative difference between the morphs in r_{MF}.

Genetic correlations between the female morphs (r_{AI}, Table 3C) differed from zero but were significantly smaller than unity. This is consistent with both the observed morphological differences between the female morphs (Abbott and Svensson, 2008; Abbott and Gosden, 2009) and with the morph-specific variation in intersexual genetic correlations found here. Previous analysis of laboratory-raised females has shown that female morphology is dependent on individual morph only, with no effect of maternal morph on the morphology of her female offspring (Abbott and Svensson, 2008). Such a pattern could not be observed if the genetic correlations between the morphs were extremely high. It is also unlikely that morph-specific intersexual genetic correlations could evolve if genetic integration between morphs was too extreme.

Since Androchrome females are often considered to be male mimics (e.g. Cordero *et al.*, 1998), and are morphologically more similar to males than Infuscans females (Abbott and Gosden, 2009), we hypothesized that this could have an effect on the magnitude of intersexual genetic correlations in this morph. Indeed, Androchrome females had more positive intersexual genetic correlations than Infuscans females and a higher mean r_{MF} (Table 3B). This is what we would expect if Androchromes benefit from more male-like morphology, although whether this is due to correlational selection for more effective male mimicry or pleiotropic effects of the morph locus is unknown. Molecular phylogenetic work on *Ischnura* and *Enallagma* species indicates that female polymorphism has evolved multiples times, and that there is weak evidence that male mimics and/or blue coloration are ancestral in these groups (Fincke *et al.*, 2005). This, in combination with the low intersexual genetic correlations in Infuscans females, suggests that repeated breakdown of such correlations may be possible. Conversely, high intersexual genetic correlations in Androchromes might thus have been adaptively maintained over a number of speciation events, due to the advantages of morphological (Abbott and Svensson, 2008; Abbott and Gosden, 2009) and behavioural (Van Gossum *et al.*, 2001) similarity to males as a way to reduce male mating harassment (Svensson *et al.*, 2005). Higher r_{MF}

in Androchromes is apparently mostly due to an increased covariance between mothers and sons (Table 4, Fig. 1). This is a particularly interesting result since there is no uncertainty regarding the relationship between mothers and sons. Low covariances between fathers and their offspring (Fig. 1) could suggest that the males captured in copula with females in this study did not actually fertilize most of the eggs, although father–offspring heritabilities were similar in magnitude to mother–offspring heritabilities for the entire data set (see Methods), which speaks against this interpretation. In addition, Infuscans females also had very low covariances with their offspring of both sexes (Fig. 1), despite the fact that they are known (not just assumed) to be related to their offspring.

The magnitude of intersexual genetic correlations can be influenced by sex-specific modifiers, sex-linkage, gene duplication followed by evolution of sex-specific expression of each locus (Rice and Chippindale, 2001; Chenoweth *et al.*, 2008), and sex-specific maternal effects (Svensson *et al.*, 2009b). Morph-specific variation in intersexual genetic correlations could therefore be due to any of these mechanisms in combination with physical linkage between loci for the morphological traits and the female morph locus, morph-specific maternal effects or pleiotropy of the morph locus. We cannot at present distinguish between these alternatives, although perhaps sex-specific maternal effects could account for the increased covariance between Androchromes and their sons relative to Infuscans females (Fig. 1). However, more research is necessary before we can conclude which mechanism(s) have produced morph-specific intersexual genetic correlations in *I. elegans*. In any case, intralocus sexual conflict and an analogous type of 'intralocus morph conflict' seem to have been partly resolved in *I. elegans*, once again highlighting the similarities between the evolution of sexual dimorphism and of intra-specific polymorphism (Abbott and Svensson, 2008).

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