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Ontogeny of sexual dimorphism and phenotypic integration in heritable morphs

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Abstract In this study we investigated the developmental basis of adult phenotypes in a non-model organism, a polymorphic damselfly (*Ischnura elegans*) with three female colour morphs. This polymorphic species presents an ideal opportunity to study intraspecific variation in growth trajectories, morphological variation in size and shape during the course of ontogeny, and to relate these juvenile differences to the phenotypic differences of the discrete adult phenotypes; the two sexes and the three female morphs. We raised larvae of different families in individual enclosures in the laboratory, and traced morphological changes during the course of ontogeny. We used principal components analysis to examine the effects of Sex, Maternal morph, and Own morph on body size and body shape. We also investigated the larval fitness consequences of variation in size and shape by relating these factors to emergence success. Females grew faster than males and were larger as adults, and there was sexual dimorphism in body shape in both larval and adult stages. There were also significant effects of both maternal morph and own morph on growth rate and body shape in the larval stage. There were significant differences in body shape, but not body size, between the adult female morphs, indicating phenotypic integration between colour, melanin patterning, and body shape. Individuals that emerged successfully grew faster and had different body shape in the larval stage, indicating internal (non-ecological) selection on larval morphology. Overall, morphological differences between individuals at the larval stage carried over to the adult stage. Thus, selection in the larval stage can potentially result in correlated responses in adult phenotypes and vice versa.

Keywords Alternative phenotypes · Antagonistic selection · Complex life-cycle · Correlational selection · Mimicry · Sexual conflict

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Recent years have witnessed an increased interest in the relationship between development and phenotype, and the problem of how integrated phenotypes evolve (West-Eberhard 2003; Pigliucci and Preston 2004). This problem is particularly interesting in the context of heritable phenotypic polymorphisms, in which distinct alternative phenotypes maintain their integrity and multitrait differences, despite being controlled by, in many cases, only one or a few genetic loci (Sinervo and Lively 1996; Shuster and Sassaman 1997; Sinervo et al. 2000; Svensson et al. 2001, 2005; Leimar 2005). There are many conceptual similarities between the persistence of such multiple alternative phenotypes, or morphs, and the evolution of gender differences and sexual dimorphism. Research on sexual size dimorphism has recently focused on its developmental origins. Investigation of how the sexes differ in growth rates and development time has shown that these factors can result in either the enhancement or suppression of adult dimorphism (Badyaev et al. 2001b; Badyaev 2002).

In addition, recent theoretical work has suggested that the evolution of sexual dimorphism or heritable polymorphism may be as likely an outcome of disruptive selection as the splitting and evolutionary branching of a population into different species (Bolnick and Doebeli 2003). In both cases, intraspecific divergence between phenotypes is constrained by the process of genetic recombination and genetic correlations between sexes or morphs (Rice and Chippindale 2001; Sinervo and Svensson 2002).

Although evolutionary developmental biology (''evo-devo'') is a rapidly growing discipline, most research in this area is still focused on classical model organisms such *Drosophila* and *Danio* (Arthur 2002). Relatively little work has been performed using non-model organisms in ecologically relevant contexts, which has consequently stimulated a recent interest in ''ecological and evolutionary developmental biology'', or ''eco-evo-devo'' (Gilbert 2001). Here we present the results from a study on the links between larval development and adult phenotype in a non-model organism, a polymorphic damselfly. Genetic colour polymorphism is very common in damselflies but is also present in many other taxa, so our study should have implications beyond our particular study species.

Our study species, *Ischnura elegans*, has three female colour morphs. Previous work revealed differences between the adult female morphs in fecundity (Svensson et al. 2005; Svensson and Abbott 2005) and emergence time (Abbott and Svensson 2005). The female morphs in *I. elegans* are maintained by frequency-dependent male-female mating interactions, in which a morph's fecundity decreases as it becomes more common in the population (Svensson and Abbott 2005). This effect arises because males are thought to form a search image towards common female morphs, which leads to a form of apostatic selection in which common morphs suffer disproportionately from excessive male mating harassment (Fincke 2004; Svensson et al. 2005). Although researchers have suggested that there may also be differences between the morphs in the larval stage (Cordero 1992a; Cordero et al. 1998), we are not aware of any studies by other researchers that have investigated this possibility. Differences in emergence and development time between the morphs (Abbott and Svensson 2005) imply that there should be morph-related differences expressed in the larval stage. This motivated us to investigate the differences in larval growth rate and body shape and their links to phenotypic differences in the adult stage, and hence evidence for phenotypic integration between growth rate, shape and colour of the morphs (phenotypic integration, as defined by Pigliucci (2003), is ''the pattern of

functional, developmental and/or genetic correlation (however measured) among different traits in a given organism''). We also present data on the ontogeny of sexual dimorphism in this species. One of our goals with this study is to integrate the study of sexual dimorphism with the study of the developmental origins of heritable morphs, a synthesis that is clearly needed and in which only the first steps have recently been taken (Badyaev 2002; West-Eberhard 2003).

Materials and methods

Study species

Ischnura elegans is a small species of annual damselfly that can be found in ponds set in open landscapes across Europe from southern Sweden to northern Spain (Askew 1988). Adult females lay eggs in the summer which hatch after several weeks and overwinter as larvae, emerging as adults the following summer. Although males are monomorphic, adult female *I. elegans* are trimorphic. One of the morphs, the Androchrome (A), is blue and black like a male, with male-like black patterning on the thorax, and is considered to be a male mimic (Cordero et al. 1998). The other two morphs, Infuscans (I) and Infuscans-obsoleta (IO), are more cryptic and are green/brown and black (Askew 1988). Of these two, Infuscans females have black patterning on the thorax similar to males and Androchrome females, while Infuscans-obsoleta females have a unique and less extensive black patterning on the thorax.

The development of the female morphs of *I. elegans* is controlled by a single locus with three alleles, similar to the closely related species, *I. graellsii* (Cordero 1990; Sánchez-Guillén et al. 2005). The three alleles form a dominance hierarchy, with the A-allele being dominant to the I- and IO-alleles, the I-allele recessive to the A-allele but dominant to the IO-allele, and the IO-allele recessive to both the other alleles (A > I > IO, Sánchez-Guillén et al. 2005). Although larvae of both sexes and adult males all carry the morph alleles, the colour morphs are only expressed in adult females, hence this is both a stage- and sex-limited polymorphism.

Morphological measurements

We collected eggs from damselflies from a natural population, Vombs Vattenverk, outside Lund, in southern Sweden in the summer of 2002. We intended to collect eggs from this population only, but it proved impossible to obtain a balanced data set in this way, due to insufficient numbers of the rarest morph (Infuscans-obsoleta). Because of this, some clutches of eggs (14 out of a total of 81 clutches) came from females captured at some of the other 13 populations we have investigated (see Svensson et al. 2005; Lomma, Hofterupssjön, Höje å 6, Höje å 7, Höje å 14, Flyinge 30A3, and Genarp).

Mature females of all three morphs were brought back to the laboratory and placed in ovipositoria, small containers with damp filter paper at the bottom. After 48 h the females were removed and the eggs stored in water until they hatched. After hatching, larvae were transferred to large plastic containers and fed with brine shrimp (artemia) daily. We transferred up to ten larvae from each family to individual enclosures within the plastic containers approximately one month after

hatching, in order to prevent cannibalism. If more than 10 individuals from the same family were available, the extra individuals were kept but are not included in the analysis of growth trajectories. The individual enclosures contained wooden perches for damselflies to crawl up during emergence.

Larvae were kept under a constant temperature and light regime (temperature: 17°C, light regime: 12:12) and were maintained in the lab until emergence next spring (2003). Individuals in the lab emerged several months earlier than individuals in the field (in January-May of 2003, rather than May-August), which is probably an effect of temperature rather than photoperiod (de Block and Stoks 2003). Though temperature affects overall timing of emergence, it does not appear to affect the relative emergence times of the morphs, the sexes, or their final size and shape, since a repetition of the same experiment the following year using two different temperature treatments (12°C and 21°C) did not show any significant effects of temperature on these measurements (Abbott, unpublished data). Once they had been transferred to the individual containers, each larva was given a unique identification number and measured under a light microscope once every 3–4 weeks until emergence. We measured total length (excluding gills), abdomen length, thorax width, width of the 4th segment of the abdomen (S4), and wing pad length (because damselflies are not holometabolous wing development begins in the larval stage), and also determined the sex of the larva by examination of the underside of the abdomen. Damselfly larvae go through several instars before reaching maturity and therefore grow in stages. This means that some individuals might not have reached the next instar between measurement times and should therefore have remained the same size. In a few cases size measurements decreased slightly between measurement times. We then assumed that this was due to measurement error, and took the average of both these measurements.

Adults were measured and, in the case of females, marked for identification and placed in $50 \times 50 \times 50$ cm insectaria containing water and *Drosophila* until their morph could be determined (no more than 25 females were housed in an insectarium at a time). We measured the same traits in adults as in larvae (total length, abdomen length, thorax width, S4 width, and wing length).

Statistics

Principal components analysis was performed on larval measurements, and the first two components were found to be suitable for further analysis. After the larvae had been moved into the individual enclosures we started recording individual mortality.

Ontogenetic changes in size and shape (PC1 and PC2) were investigated using repeated measures (PROC MIXED, SAS, Littell et al. 1996). The correct covariance structure was determined by comparing the Akaike Information Criterion (AIC). We investigated the effects on PC1 and PC2 of the fixed factors Maternal morph and Sex in all individuals, and of Own morph in females only. We also investigated whether there was any difference in developmental trajectories between individuals that managed to emerge successfully and those that did not. Family was included as a random factor in all analyses, except of the effect of own morph on PC1, to control for non-independence of siblings (Fry 1992). It was impossible to include Family as random factor in the analysis of the effect of Own morph on PC1, probably because this subset of the data was too unbalanced, so in this case, Family was included as a fixed factor instead. Two-way interactions between all factors

(except Sex*Morph since males are monomorphic and Morph*Emergence since we could not determine morph for females that died in the larval stage) were also tested but this did not change the results, so for simplicity interaction effects will not be presented here. An analysis of the effect of Maternal morph on PC2 for males only was also carried out, to see whether differences between offspring of the morphs were due to biased sex ratios.

We also looked at the effects of Sex, Maternal morph, Own morph, and whether the individual emerged successfully on morphology in the last instar using a mixed model with Family as a random factor.

We analysed the probability of emerging according to Sex and Maternal morph with Family as a random factor using a generalized linear model (GLIMMIX macro in SAS, Littell et al. 1996) with binomial error and logit link function. This was done to investigate if differences between individuals that emerged and those that did were possibly confounded by differences in survival rates between the sexes, between offspring of the female morphs, or between families.

A separate principal components analysis was performed on the lab-raised adults, and again, the first two components were selected for further analysis. Mixed model analyses with Family as a random factor nested within Maternal morph were performed in SAS (Littell et al. 1996). Family was nested within Maternal morph because each Family can by definition only have one value for Maternal morph, precluding any interaction between these two factors (Abbott and Svensson 2005). We analysed the effects of Sex and Maternal morph on PC1 and PC2 in all individuals, and the effects of Maternal morph and the individual's Own morph in females only. All analyses included interactions between fixed factors. Post-hoc comparisons of least square means were carried out for significant effects.

To investigate if any differences between groups were confounded by population effects, we included Population as a random factor in all analyses, both of larval growth trajectories and adult morphology. Population was never significant (all P-values > 0.10) and did not affect our results, so we only present models here that do not include Population as a factor.

Finally, we calculated phenotypic correlations between traits in the final larval instar and the same trait in the adult stage, using STATISTICA (Statsoft 2004).

Results

Mortality

Mortality was relatively modest; 28% of all individually tracked larvae died (227/806 individuals). By plotting a histogram of wing pad lengths we were able to identify when individuals had reached the last instar (in this case, when wing pad length was greater than 3.5 mm (Benke 1970)). Most of these individuals (174/806, or 21%) died when in early instars, not long after being moved into the individual enclosures, probably as a result of the changed environmental conditions. These individuals were excluded from all further analyses. The remainder (53/806, or 7%) died in the last instar, close to or during emergence. We believe that it is unlikely that individuals that had survived several months after being moved into the individual enclosures suddenly died because of the conditions in the lab, and so we assumed that this later mortality was related to problems during emergence. Probability of

emerging was not related to Maternal morph ($F_{2,\ 618} = 0.12$, $P = 0.8884$), Sex ($F_{1,\ 628} = 0.68$, $P = 0.4110$), or Family ($F_{78,\ 628} < 0.01$, $P > 0.99$) so differences between individuals that emerged and those that did not are not a result of differential mortality between these groups.

Larval morphology

The principal components analysis of larval morphology indicated that PC1 was a measure of overall size, which accounted for most of the variation in morphology (96%). There was also a minor component of the variation (2.7%) which was related to variation in shape, such that positive values of PC2 indicate a longer abdomen and shorter wings, while negative values indicate a shorter abdomen and longer wings (Table 1). The last three PCs accounted for less than 1% of the variation each. Although PC2 accounted for a small part of the total variation, this is probably due to the nature of the data set (a growth series). According to Jackson (1991), in cases where the first principal component accounts for an overwhelming part of the variation in the data it may still be appropriate to include other PCs in the analysis as long as they are informative, i.e. the PC has an eigenvalue unequal to all subsequent PCs. Since the difference in eigenvalue between PC2 and PC3 is almost three and a half times greater than the difference in eigenvalue between PC3 and PC4 (0.093 versus 0.027), we believe that PC2 is actually capturing an important and informative, if relatively small, part of the total variation. In addition, PC2 in the larval and adult stages both indicate a negative relationship between wing length and abdomen length, as does PC2 in an analysis of morphology of field-caught adults (Abbott, 2006), all of which suggests that the pattern seen in PC2 in the larval stage is informative.

We found significant effects of all factors tested on body size (PC1) and body shape (PC2). In these analyses, significant effects indicate differences between the equations of the best-fit lines which describe the data. Main effects correspond to differences in intercept, the factor*time interactions to differences in slope, and the factor*time^2 interactions to differences in curvature (Littell et al. 1996). For body size, we found that females had a higher growth rate than males, that offspring of Infuscans-obsoleta females had a higher growth rate than the offspring of the other two morphs, and that Androchrome females had a slightly higher growth rate than females of the other two morphs (Table 2, Fig. 1A–C). Individuals that managed to emerge had a higher growth rate than individuals that did not emerge (Table 2, Fig. 1D). Females in the last instar were significantly larger than males in the last instar ($F_{1,\ 632} = 141.70$, $P < 0.0001$), Androchrome females were significantly larger

Table 1 Factor loadings for PC1 and PC2 in the larval stage

Measurement	Loading PC1	Loading PC2
Length	0.991	0.082
Abdomen	0.987	0.121
Thorax	0.993	0.013
S4	0.983	0.093
Wing	0.946	–0.323

PC1 is a measure of overall size, while PC2 mostly represents a trade-off in wing length and abdomen length

Table 2 Table of repeated measures analysis of effects of Sex, Maternal morph, Own morph, and Emergence on PC1 (body size) in the larval stage

Effect	Num Df	Den Df	*F*	*Z*	*P*-value
Sex ($N = 632$):					
Sex	2	185	6.46		0.0019
Sex*time	2	565	5567.42		<0.0001
Sex*time^2	2	575	169.73		<0.0001
Family	78			3.60	0.0002
Maternal morph ($N = 622$):					
Maternal morph	3	105	2.81		0.0429
Maternal morph*time	3	562	3749.10		<0.0001
Maternal morph*time^2	3	574	124.71		<0.0001
Family	77			3.54	0.0002
Own morph (females only, $N = 229$):					
Own morph	3	139	0.99		0.3973
Own morph*time	3	203	1395.54		<0.0001
Own morph*time^2	3	199	47.45		<0.0001
Family	76	53.3	1.91		0.0066
Emergence ($N = 628$):					
Emergence	2	232	3.17		0.0440
Emergence*time	2	543	5586.27		<0.0001
Emergence*time^2	2	553	172.05		<0.0001
Family	78			3.51	0.0002

Family was included as a random factor in all analyses, except Own morph, where it is a fixed factor (see text). A significant effect of the factor indicates significant differences in the intercepts of the trajectories, a significant interaction between the factor and time indicates significant differences in the slope of the trajectories, and a significant interaction between the factor and time^2 indicates significant differences in the curvature of the trajectories. For fixed effects (Maternal morph, Sex, Own morph, Emergence) the test statistic is *F*, for random effects (Family) it is *Z*

than Infuscans females in the last instar ($F_{2,\ 229} = 5.91$, $P = 0.0032$), and individuals that emerged successfully were larger in the last instar than individuals that did not emerge ($F_{1,\ 628} = 13.11$, $P = 0.0003$; Table 4).

For body shape, we found that males start off with shorter abdomens and longer wing pads than females, but that they end up with longer abdomens and shorter wing pads (Table 3, Fig. 2A). We also found that offspring of Infuscans-obsoleta females have longer abdomens and shorter wing pads than the offspring of the other two morphs (Fig. 2B). This pattern held even when only males were included in the analysis (quadratic time effect of Maternal morph: $F_{2,\ 258} = 318.27$, $P < 0.0001$; pattern is the same as in Fig. 2B), so this reflects a real effect of Maternal morph on offspring morphology which cannot simply be a result of biased sex or morph ratios in offspring. Individuals that managed to emerge initially had shorter abdomens and longer wing pads (lower values of PC2) than individuals that did not, with the reverse pattern later in development (Fig. 2D). This was also evident in the last instar, where individuals that emerged successfully had longer abdomens and shorter wing pads than individuals that did not emerge ($F_{1,\ 628} = 19.43$, $P < 0.0001$; Table 4). This suggests the existence of internal selection on body shape. There was also an effect of Own morph on body shape, with rank order of the different morphs changing several times over development (Fig. 2C). The difference between the morphs in the final instar approached significance, with Androchrome females

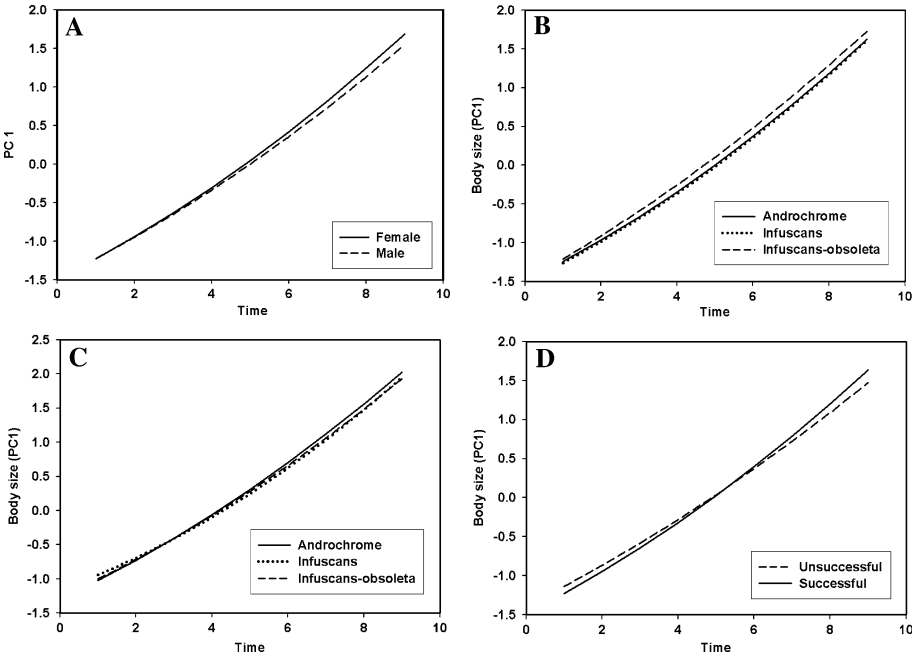

Fig. 1 The predicted effects of different factors on body size (PC1) in the larval stage. (**A**) The effect of Sex on body size. Females have a higher growth rate than males. (**B**) The effect of Maternal morph on body size. Offspring of Infuscans-obsoleta females have a higher growth rate than offspring of the other morphs. (**C**) The effect of Own morph on body size. Androchrome females have a higher growth rate than females of the other morphs. (**D**) The effect of Emergence on body size. Individuals that emerge have a higher growth rate than individuals that do not emerge, but are smaller initially

having a more male-like morphology (higher value of PC2) than the other two morphs ($F_{2,\ 229} = 2.92$, $P = 0.0560$; Table 4).

Laboratory-raised adults

Similar to the analysis of larval morphology, PCA on adult morphology resulted in PC1 as a measure of overall size which accounted for 60.1% of the variation. PC2 was found to be a measure of shape which accounted for 26.5% of the variation, where positive values indicate relatively longer abdomens, but shorter wings and narrower S4, and negative values indicate relatively shorter abdomens, with longer wings and wider S4 (Table 5). All other PCs accounted for a relatively small part of the variation (data not shown).

Males and females differed in both body size (females were larger, Table 6 and Fig. 3) and body shape (males have relatively longer abdomens, shorter wings, and narrower S4, Table 7 and Fig. 4A). There were no differences in body size between the different morphs or between the offspring of the different morphs. Females of different morphs did, however, differ in body shape. Infuscans-obsoleta females had shorter abdomens, longer wings and wider S4 (Fig. 4B). There was no difference between the offspring of the three morphs in body shape.

Table 3 Table of repeated measures analysis of effects of Sex, Maternal morph, Own morph, and Emergence on PC2 (body shape) in the larval stage

Effect	Num Df	Den Df	*F*	*Z*	*P*-value
Sex ($N = 632$):					
Sex	2	151	2.45		0.0895
Sex*time	2	546	13.82		<0.0001
Sex*time^2	2	561	1148.64		<0.0001
Family	78			4.40	<0.0001
Maternal morph ($N = 622$):					
Maternal morph	3	86	0.48		0.6943
Maternal morph*time	3	537	9.10		<0.0001
Maternal morph*time^2	3	551	728.88		<0.0001
Family	77			4.34	<0.0001
Own morph (females only, $N = 229$):					
Own morph	3	185	2.33		0.0761
Own morph*time	3	215	4.81		0.0029
Own morph*time^2	3	218	417.54		<0.0001
Family	76			3.37	0.0004
Emergence ($N = 628$):					
Emergence	2	229	0.22		0.7988
Emergence*time	2	531	20.04		<0.0001
Emergence*time^2	2	541	1217.44		<0.0001
Family	78			4.39	<0.0001

Family was included as a random factor in all analyses. A significant effect of the factor indicates significant differences in the intercepts of the trajectories, a significant interaction between the factor and time indicates significant differences in the slope of the trajectories, and a significant interaction between the factor and time^2 indicates significant differences in the curvature of the trajectories. For fixed effects (Maternal morph, Sex, Own morph, Emergence) the test statistic is *F*, for random effects (Family) it is *Z*

All phenotypic correlations between size measurements in larval and adult stages were highly significant (Table 8), indicating that morphological differences carry over between the stages. In addition, in 8 cases out of 10, the factor loading for a trait in the larval stage and in the adult stage is the same (Tables 1 and 5), suggesting that the pattern of variation in size and shape is similar in both stages.

Discussion

Sexual dimorphism and heritable polymorphism in *I. elegans* are characterized by phenotypic integration of colour and morphology (this study), and differences in development time between different phenotypes (Abbott and Svensson, 2005). In addition, development rate interacts with development time to influence size. In the sexes, size differences are enhanced by this interaction, while in the morphs, size differences are instead suppressed by the same type of interaction.

Size differences

Sexual size dimorphism in vertebrates can result from differences in development time, development rate or both these factors acting jointly (Badyaev 2002). Here we have shown that females have a higher larval growth rate than males (Fig. 1A), and

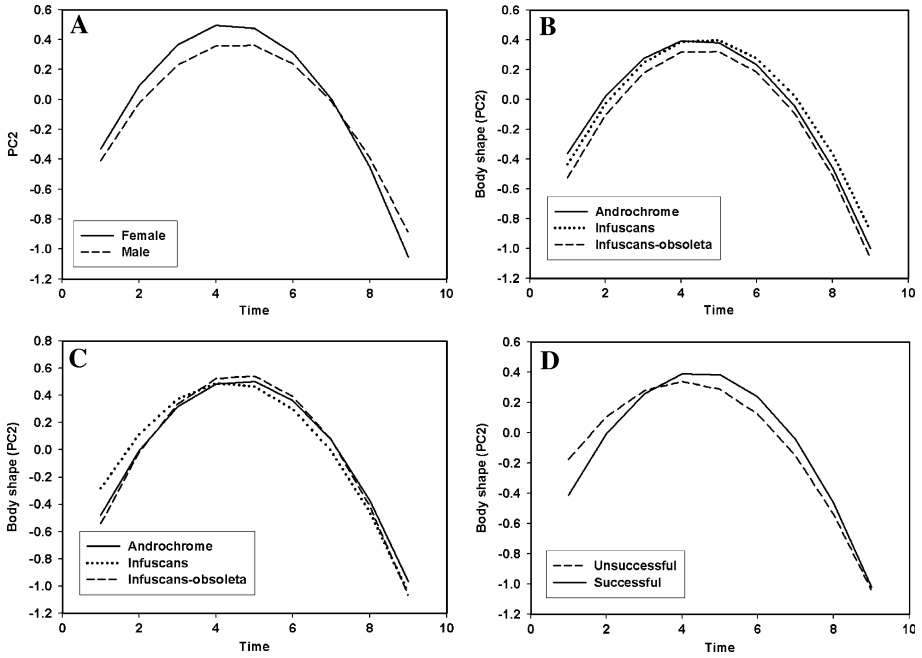

Fig. 2 The predicted effects of different factors on body shape (PC2) in the larval stage. (**A**) The effect of Sex on body shape. Males start off with longer wings and shorter abdomens (smaller values of PC2) but end up with shorter wings and longer abdomens than females (larger values). (**B**) The effect of Maternal morph on body shape. Offspring of Infuscans-obsoleta females have shorter wings and longer abdomens (higher values of PC2) than the offspring of the other two morphs. (**C**) The effect of Own morph on body shape. Rank order of the morphs changes several times throughout ontogeny. (**D**) The effect of Emergence on body shape. Individuals that emerge have longer wings and shorter abdomens (lower values of PC2) than individuals that do not emerge

were larger in the final instar (Table 4) and as adults (Fig. 3). In a previous analysis of data from the same laboratory-raised population (Abbott and Svensson 2005), we have shown that males emerged earlier than females (protandry). Thus, sexual differences in development time and development rate are acting jointly, and in the same direction, to promote sexual size dimorphism in *I. elegans*.

In contrast, for the offspring of the different morphs, development time and development rate cancel each other out with respect to size. Offspring of Infuscans-obsoleta females were found to emerge earlier than the offspring of the other morphs (Abbott and Svensson 2005), but despite this they do not differ in size as adults (Table 6). Instead, they grow faster in the larval stage (Fig. 1B), making them able to attain the same size in a shorter time. Androchrome females had a slightly higher growth rate than the other two morphs were larger in the final instar (Table 4), although the difference did not carry over to the adult stage. Since there was no competition in our experimental design, this difference could be due to differences in efficiency in obtaining and assimilating food. Adult Androchrome females have been found to be larger than the other morphs in some populations (Cordero 1992a), which was suggested to be a result of competitive differences between morphs at the larval stage. Our results indicate that pleiotropic, physiological effects of the morph locus may also be involved.

Table 4 LS means of (A) morphological measurements and PCs in the final instar according to Sex, Maternal morph, Own morph, and Emergence and (B) morphological measurements in the adult stage according to Sex and Own morph

Panel A

Sex:			
	Female	Male	
Length	15.13 (0.07)	14.70 (0.07)	
Abdomen	10.12 (0.04)	10.92 (0.04)	
Thorax	2.37 (0.007)	2.28 (0.006)	
S4	1.45 (0.005)	1.36 (0.005)	
Wing	4.47 (0.017)	4.20 (0.016)	
PC1	1.50 (0.016)	1.31 (0.016)	
PC2	–0.87 (0.032)	–0.84 (0.030)	
Maternal morph:			
	Androchrome	Infuscans	Infuscans-obsoleta
Length	14.90 (0.10)	14.83 (0.12)	14.96 (0.13)
Abdomen	10.00 (0.05)	9.99 (0.07)	10.06 (0.07)
Thorax	2.33 (0.009)	2.31 (0.011)	2.32 (0.012)
S4	1.41 (0.006)	1.40 (0.008)	1.40 (0.009)
Wing	4.34 (0.022)	4.29 (0.029)	4.35 (0.029)
PC1	1.40 (0.022)	1.37 (0.029)	1.42 (0.030)
PC2	–0.87 (0.032)	–0.83 ((0.052)	–0.85 (0.053)
Own morph:			
	Androchrome	Infuscans	Infuscans-obsoleta
Length	15.40 (0.09)	14.95 (0.11)	14.98 (0.23)
Abdomen	10.27 (0.05)	10.04 (0.06)	10.02 (0.12)
Thorax	2.39 (0.009)	2.35 (0.010)	2.37 (0.022)
S4	1.46 (0.006)	1.44 (0.006)	1.46 (0.014)
Wing	4.54 (0.022)	4.45 (0.024)	4.44 (0.052)
PC1	1.57 (0.022)	1.47 (0.025)	1.49 (0.053)
PC2	–0.82 (0.036)	–0.93 (0.040)	–0.85 (0.086)
Emergence:			
	Unsuccessful	Successful	
Length	14.37 (0.14)	14.94 (0.06)	
Abdomen	9.70 (0.08)	10.04 (0.04)	
Thorax	2.27 (0.014)	2.32 (0.006)	
S4	1.39 (0.011)	1.40 (0.004)	
Wing	4.29 (0.039)	4.33 (0.015)	
PC1	1.29 (0.034)	1.41 (0.015)	
PC2	–1.10 (0.061)	–0.84 (0.027)	

Panel B

Sex:			
	Female	Male	
Length	30.07 (0.10)	30.06 (0.09)	
Abdomen	23.54 (0.08)	23.78 (0.08)	
Thorax	2.19 (0.007)	2.09 (0.006)	
S4	0.73 (0.004)	0.62 (0.003)	
Wing	18.77 (0.06)	17.10 (0.05)	
Own morph:			
	Androchrome	Infuscans	Infuscans-obsoleta
Length	30.20 (0.13)	30.06 (0.15)	29.61 (0.30)
Abdomen	23.62 (0.11)	23.58 (0.12)	23.10 (0.25)
Thorax	2.20 (0.010)	2.18 (0.011)	2.21 (0.022)
S4	0.72 (0.005)	0.73 (0.005)	0.75 (0.011)
Wing	18.85 (0.08)	18.75 (0.09)	18.59 (0.19)

All values were calculated from mixed models with family as a random factor and are presented in the form Mean (SE). Morphological measurements (total length, abdomen length, thorax width, width of the 4th segment of the abdomen, and wing pad length) are in mm

Table 5 Factor loadings for PC1 and PC2 in laboratory-raised adults. PC1 is a measure of overall size, while PC2 mostly represents a trade-off in wing length/S4 width and total length/abdomen length

Measurement	Loading PC1	Loading PC2
Length	0.815	0.537
Abdomen	0.732	0.636
Thorax	0.874	–0.197
S4	0.630	–0.675
Wing	0.823	–0.372

Table 6 Table of mixed model analysis of effects of Sex, Maternal morph and Own morph on PC1 (body size) in the adult stage

Effect	Num Df	Den Df	*F*	*Z*	*P*-value
All individuals (N = 558):					
Maternal morph	2	73.4	0.66		0.5190
Sex	1	513	174.50		<0.0001
Maternal morph*Sex	2	513	0.03		0.9722
Family	77			4.08	<0.0001
Females only (N = 232):					
Maternal morph	2	108	0.10		0.9011
Own morph	2	217	0.59		0.5545
Maternal morph*Own morph	4	216	1.38		0.2405
Family	75			3.18	0.0007

Family was included as a random factor in all analyses. Maternal morph and Sex were included in the first analysis (all offspring), and Maternal morph and Own morph in the second (females only). For fixed effects (Maternal morph, Sex, Own morph) the test statistic is *F*, for random effects (Family) it is *Z*

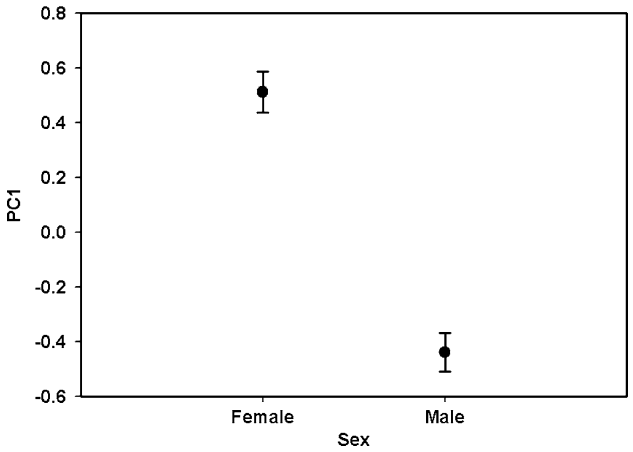

Fig. 3 Difference between males and females in body size (PC1) in the adult stage. Females are significantly larger

Shape differences

Shape differences between the sexes and the morphs were generally consistent between the different life stages. In the adult stage, males have relatively longer

Table 7 Table of mixed model analysis of effects of Sex, Maternal morph and Own morph on PC2 (body shape) in the adult stage

Effect	Num Df	Den Df	F	*Z*	*P*-value
All individuals (N = 558):					
Maternal morph	2	71.2	1.45		0.2409
Sex	1	526	510.29		<0.0001
Maternal morph*Sex	2	526	1.77		0.1705
Family	77			3.12	0.0009
Females only (N = 232):					
Maternal morph	2	74.8	0.88		0.4205
Own morph	2	207	3.09		0.0478
Maternal morph*Own morph	4	204	0.16		0.9600
Family	75			0.88	0.1905

Family was included as a random factor in all analyses. Maternal morph and Sex were included in the first analysis (all offspring), and Maternal morph and Own morph in the second (females only). For fixed effects (Maternal morph, Sex, Own morph) the test statistic is *F*, for random effects (Family) it is *Z*

abdomens, shorter wings, and narrower S4 (Fig. 4A) than females. This is consistent with their shape in the final instar, where males have longer abdomens and relatively shorter wing pads than females (Table 4). Similarly, the Infuscans-obsoleta morph was the most divergent morph in both stages, although in the adult stage this was evident as an effect of the female's Own morph (Fig. 4B), while in the larval stage it was due to the effect of Maternal morph (Fig. 2B, comparing parental and offspring traits is a standard quantitative-genetic approach; see Abbott and Svensson, 2005 for details).

Both size and shape differences seem to have additive genetic components, as indicated by the significant effects of the factor Family on both PC1 (Tables 2 and 6) and PC2 (Tables 3 and 7). Body length has previously been demonstrated to be heritable in a related species (Cordero 1992b). Our findings that most of this variation is aligned along the size axis with less variation in shape is consistent with many other quantitative-genetic studies on other organisms (Schluter 1996). The phenotypic correlations found here also confirm that larval and adult size and shape are related (Table 8), which has previously been shown for size (Harvey and Corbet 1985; Banks and Thompson 1987; Cordero 1992b). In the closely related damselfly genus *Enallagma*, larval phenotypic traits influenced by selection imposed by different aquatic predators such as fish or dragonfly larvae may show a correlated response to selection on reproductive traits in the adult stage (Stoks et al. 2003; Stoks et al. 2005).

Sexual dimorphism

Adult males of *I. elegans* are both smaller than females and different in shape, as well as being monomorphic for colour (in contrast to the colour polymorphic females). The size difference between the sexes is probably a result of selection for protandry (earlier emergence of males), since males engage in scramble competition for females. Previous field studies have shown that small males may have higher mating success in some populations (Cordero et al. 1997; Carchini et al. 2000). For females, fecundity is likely to be more influenced by body size than by timing of emergence (Cordero 1991; Morbey and Ydenberg 2001). Thus, sexual size dimor-

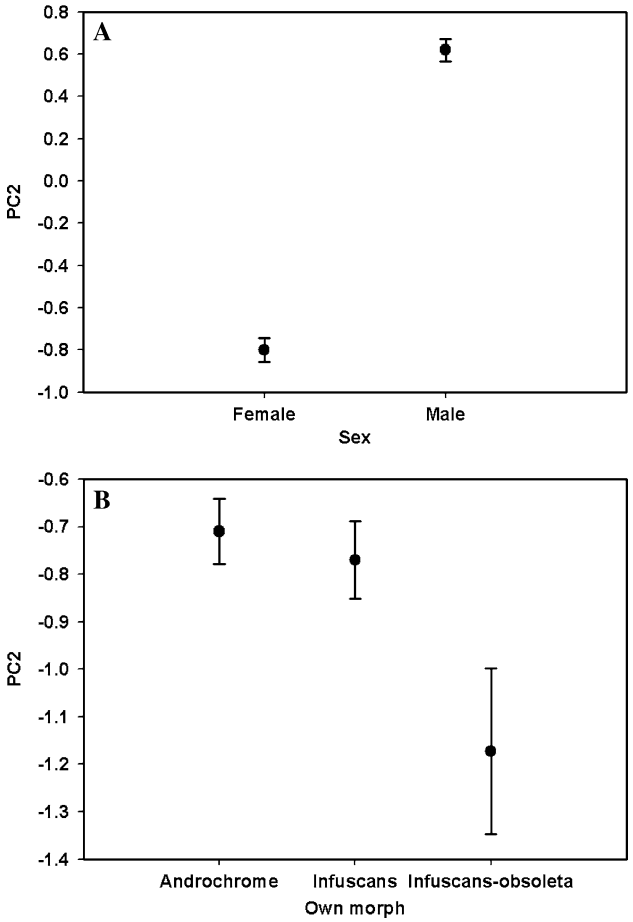

Fig. 4 Differences in body shape (PC2) in the adult stage between (A) Males and females. Males have relatively longer abdomens, shorter wings, and narrower S4 than females. (B) Females of different morphs. Infuscans-obsoleta females were significantly different ($P < 0.05$) from Androchrome and Infuscans females, with relatively shorter abdomens, longer wings and wider S4

Table 8 Table of phenotypic correlations between larval and adult traits

Trait	*r*	*P*-value
Length	0.5021	< 0.001
Abdomen	0.4358	< 0.001
Thorax	0.6527	< 0.001
S4	0.5864	< 0.001
Wing	0.7696	< 0.001

Only correlations between the same trait measured in both stages in the same individual are included (i.e. larval body length in the last instar correlated with adult body length, larval abdomen length in the last instar with adult abdomen length, etc.)

phism in this species may result from sexually antagonistic selection on body size, with different size optima for males and females (Rice and Chippindale 2001). The shape differences between the sexes should reflect adaptive differences arising from

gender-specific reproductive roles. Males must have relatively long abdomens for completion of the wheel position during mating (Corbet 1999) and females may have wider abdomens than males in order to accommodate the ovaries. The presence of the ovaries implies that females should be heavier than males of the same length, which may in turn select for longer wings.

We also note that the maternal morphs also influence the shape of their monomorphic sons (Table 3 and Fig. 2B). An analysis of the effect of Maternal morph on PC2 in only males results in the same pattern as seen in Fig. 2B, with male offspring of Infuscans-obsoleta females having the most male-like shape in the larval stage. This may have some implications for ontogenetic sexual conflict between loci affecting overall shape and the morph locus. We have previously argued in a similar vein that there is a conflict between loci for early emergence favouring male protandry and the morph-locus which also influences development time in both males and females (Abbott and Svensson 2005).

Phenotypic integration

The fact that the female morphs in *I. elegans* differ in colour (Askew 1988), shape and development rate (this study), as well as development time (Abbott and Svensson 2005) and fecundity (Svensson et al. 2005; Svensson and Abbott 2005), suggests that suites of phenotypic traits are integrated in these morphs. This has some similarities to the adaptive phenotypic integration documented for male secondary sexual characters in several avian taxa, which are thought to be promoted by correlational selection for optimal character combinations (Badyaev et al. 2001a; Badyaev 2004; McGlothlin et al. 2005). Multi-trait differences between the morphs could have been caused by maternal effects, pleiotropy, or linkage disequilibrium (Lynch and Walsh 1998) due to physical linkage between loci for colour and morphology or which is built up in each generation by correlational selection (Brodie III 1992). The data in this study do not allow us to distinguish between these different explanations for the persistence of multi-trait differences between these morphs.

The general pattern and direction of morph-specific differences in *I. elegans* are consistent with the hypothesis that Androchrome females are male mimics, because they have both male-like melanin patterning, male-like blue coloration, male-like behaviour (Van Gossum et al. 2001) and they are also more male-like in shape (i. e. high value of PC2, cf. Fig. 4). It is possible that these striking and multiple phenotypic similarities between Androchromes and males are simply non-adaptive pleiotropic effects of the allele producing male-like coloration. However, the observed pattern is certainly also consistent with selection to improve male mimicry in Androchromes either through direct selection on shape, or indirectly via selection for more male-like behaviour, such as flight or movement patterns, or as a response to avoiding male mating harassment. For instance, morph-differences in relative wing to abdomen length (i. e. PC2, see Fig. 4B) may affect flight speed or manoeuvrability, and thereby success in escaping unwanted male mating harassment and mating attempts.

Male mating harassment in Ischnurans is likely to substantial since females mate with multiple males (Cooper et al. 1996) but only require one insemination to produce as many fertile eggs as females that have mated several times (Sirot and Brockmann, 2001), and more mating attempts are initiated then are carried out (T. Gosden and E. I. Svensson, unpublished data). This harassment may select for

different phenotypic female optima, so that females can avoid such harassment by either becoming a more or less perfect male mimic (i. e. Androchromes) or by developing a divergent phenotype in colour and shape (i. e. Infuscans-obsoleta) or by becoming so different that it falls outside the usual range of female phenotypes encountered by males. Interestingly, Infuscans-obsoleta is also the morph that is found least frequently in copula in the field, relative to their frequency in the population (Svensson et al. 2005).

These adaptive explanations for the phenotypic integration in female morphs are consistent with both models and data that that indicate intraspecific genetic diversification is an expected outcome of male mating harassment (Gavrilets and Waxman 2002), particularly if males have visual or other perceptive constraints that force them to develop a search image for only one female morph at a time (Fincke 2004; Svensson et al. 2005). Such intraspecific divergence has two possible outcomes: it could subsequently promote speciation, or constrain it by eliminating selection pressures for additional divergence through the formation of stable female genetic clusters (polymorphism; Svensson et al. 2005).

Finally, although differences between these morphs in shape are relatively modest relative to interspecific differences (Table 4; Fig. 4B), we note that recombination is expected to limit intraspecific divergence between sympatric morphs of this kind (Sinervo and Svensson 2002). Hence, although the fitness optima of the morphs may differ substantially, realized (observed) differences in nature between morphs will be more moderate in magnitude, due to the constraining effects of recombination (Table 4; Fig. 4B).

Fitness consequences of variation in size and shape: internal selection on morphology?

We found evidence for fitness consequences on morphology in the larval stage, since individuals that managed to emerge successfully differed in both size and shape (PC1 and PC2) from those that did not. Surprisingly, individuals that emerged started off smaller in size than those that did not (Fig. 1D). There are two possible explanations for this pattern, antagonistic pleiotropy and competition. In antagonistic pleiotropy, alleles with positive effects early in development have negative effects later in development (Rose 1982). Alternatively, there could be differences in competitive ability which are the result of a trade-off between growth rate while under intraspecific competition and growth rate when solitary, since larvae were not moved to individual enclosures until a few weeks after hatching. Such trade-offs between growth rate under crowded an non-crowded conditions have indeed been documented previously in laboratory selection experiments of *Drosophila* (Mueller and Ayala 1981; Mueller 1988; Borash et al. 1998).

Since individuals that emerged successfully differed in body shape from those that did not, this suggests that there is selection on body shape in the larval stage. This type of selection could contribute to the build-up of linkage disequilibrium in the female morphs (see above). Individuals that emerged had shorter abdomens and longer wings than those that did not, so there appears to be some sort of internal (''non-ecological'') selection on shape. Internal selection refers to selection that acts on organismal traits independently of ecology (Schwenk and Wagner 2001). Internal selection caused by developmental problems is more likely in this laboratory study in which predators and other ecological agents of selection can be excluded as

mortality causes. The fact that this type of internal selection appeared to favour shorter wings (see Results) is particularly interesting and may indicate that there may be developmental fitness costs of long wings that may counteract selection for longer wings or larger size at the adult stage (Kingsolver and Pfennig 2004). The relevance of such selection in the field is unknown, but could be important if mortality due to other causes (such as predation) is random with respect to an individual's ability to emerge successfully.

Conclusions

We have found evidence of phenotypic integration of many traits in the female morphs, such as colour pattern, morphology, developmental rate (this study), development time (Abbott and Svensson 2005) and fecundity (Svensson et al. 2005; Svensson and Abbott 2005). These and other results reveals the similarities between the development of morphological differences of heritable morphs in *Ischnura elegans* and the development of sexual dimorphism in both this insect and vertebrate species (Badyaev 2002). Both these phenomena can be analysed and understood in terms of the interactive effects of developmental rate and development time, two factors which can enhance or counteract each other during the course of development. We are currently investigating sexual dimorphism and phenotypic integration in field-caught adults, genetic correlations and heritability of morphological traits, and are also analyzing larval morphology using geometric morphometric techniques. Other interesting questions for further research include the relative importance of maternal effects, pleiotropy, and linkage disequilibrium (of linked or unlinked loci) in producing morph-related differences, and the effect of competition on development of adult phenotypes.

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