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Phenotypic and genetic variation in emergence and development time of a trimorphic damselfly

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sex-limitation.

Abstract

Although colour polymorphisms in adult organisms of many taxa are often adaptive in the context of sexual selection or predation, genetic correlations between colour and other phenotypic traits expressed early in ontogeny could also play an important role in polymorphic systems. We studied phenotypic and genetic variation in development time among female colour morphs in the polymorphic damselfly *Ischnura elegans* in the field and by raising larvae in a common laboratory environment. In the field, the three different female morphs emerged at different times. Among laboratory-raised families, we found evidence of a significant correlation between maternal morph and larval development time in both sexes. This suggests that the phenotypic correlation between morph and emergence time in the field has a parallel in a genetic correlation between maternal colour and offspring development time. Maternal colour morph frequencies could thus potentially change as correlated responses to selection on larval emergence dates. The similar genetic correlation in male offspring suggests that sex-limitation in this system is incomplete, which may lead to an ontogenetic sexual conflict between selection for early male emergence (protandry) and emergence times associated with maternal morph.

Introduction

Colour polymorphisms are found in many different taxa, such as birds (Galeotti *et al.*, 2003; Roulin *et al.*, 2003), amphibians (Hoffman & Blouin, 2000), fish (Munday *et al.*, 2003), reptiles (Sinervo *et al.*, 2000), plants (Schemske & Bierzychudek, 2001; Turelli *et al.*, 2001) and insects (Forsman & Appelqvist, 1999; Mallet & Joron, 1999), and have become classical study systems among evolutionary biologists and ecologists. Models for the maintenance of multiple morphs typically focus on negative frequency-dependent selection resulting from intra- or interspecific biotic processes such as predation (Cain & Sheppard, 1954; Cook, 1998; Davison, 2002) or sexual selection (Nielsen & Watt, 2000). Rare morphs have an advantage in systems where predators form a search image and prey more heavily on the most common morph (Allen, 1988; Weale *et al.*, 2000; Shigemiya, 2004). Rare male morphs may have an advantage under sexual selection, as in the side-blotched lizard *Uta stansburiana*, where each of the three male throat colour morphs has highest reproductive success when at low frequency (Sinervo & Lively, 1996). Sex-limited polymorphisms are usually assumed to be maintained via sexual selection and male–female interactions (Svensson *et al.*, 2005).

Most models also implicitly assume that colour is only subject to selection in the context of sexual selection or predation. It is, however, possible that there are other phenotypic differences between morphs which are unrelated to colour but which are also under selection, e.g. physiological traits that are expressed during earlier parts of the life-cycle. Examples of such traits that are correlated with colour come from studies of colour polymorphic insects and reptiles, in which differences between morphs in traits as diverse as developmental timing, fecundity and disease resistance have been documented (Fahmy & Fahmy, 1959; Cook & Jacobs, 1983; Svensson *et al.*, 2001a,b, 2002; Wilson *et al.*, 2001; True, 2003). When colour morphs are genetically

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correlated with other traits, as in the cases cited above, selection on such other traits can potentially result in a correlated response in morph frequencies.

Here, we present data from a field and laboratory study of a trimorphic damselfly, aimed at investigating the links between adult colour, larval development and emergence time. Female-limited polymorphisms are found in many species of damselflies (Cordero, 1992; Fincke, 1994; Andrés & Cordero Rivera, 2001; Sirot *et al.*, 2003; Wong *et al.*, 2003), dragonflies (Corbet, 1999) and butterflies, as well as some species of birds (Bleiweiss, 1992; Roulin *et al.*, 2003). Species of damselfly with female-limited colour polymorphism usually have one morph that resembles a male, so-called Androchrome females (Corbet, 1999). It has been suggested that Androchrome females may have a selective advantage in that they can, as male mimics, avoid costly male mating harassment and superfluous matings. As differences have been found between colour morphs of other insects in development time (Cook & Jacobs, 1983; Ahnesjö & Forsman, 2003), we investigated if there was any evidence for a similar relationship in the polymorphic blue-tailed damselfly (*Ischnura elegans*). The results in this study suggest that female colour morphs are both phenotypically and genetically correlated with larval development time and emergence date.

Methods

Study species

Ischnura elegans is a small damselfly in which females are trimorphic and males are monomorphic (Askew, 1988). The males' abdomen is black, except for the eighth segment, which is blue, and they have a blue thorax with three longitudinal black stripes. The Androchrome (A) morph has the same colouration and patterning as a male, and is therefore considered to be a male mimic. The two other morphs are often grouped together as Gynochrome morphs (Gynochrome = 'female-coloured'), as their colouration is green (*Infuscans*) or brown (*Infuscans-obsoleta*) and potentially more cryptic (Cordero *et al.*, 1998). Though the *Infuscans* (I) morph has the same black patterning as males and Androchromes, the *Infuscans-obsoleta* (IO) morph lacks two of the black stripes on the thorax (the humeral stripes) and retains only the central stripe (Askew, 1988). *Infuscans-obsoleta* females can be identified from first emergence because of their unique pattern of black colouration, while Androchromes and *Infuscans* females are both purple when immature, and impossible to distinguish until they achieve their mature colouration.

The development of the female morphs of *I. elegans* is controlled by a single locus with three alleles, as are the corresponding morphs in the sister species, *I. graellsii* (Cordero, 1990; Sánchez-Guillén *et al.*, 2005). The three alleles of the morph locus form a dominance hierarchy, with the A-allele 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)]. *I. elegans* has one generation per year.

Emergence of females in the field

Fourteen populations outside Lund, in southern Sweden (Fjelie, Flyinge 30A1, Flyinge 30A3, Genarp, Gunnesbo, Habo, Hofterups, Höje Å 14, Höje Å 6, Höje Å 7, Lomma, Lund South, Vallby Mosse and Vombs Vattenverk), were visited between the years 2000 and 2003. In each of these populations, damselflies were surveyed regularly over the season (late May/early June to early/mid August) to determine morph frequencies. We captured 2621 females in total, but excluded immature females from the analysis, resulting in a final sample size of 2127 females. Captured females were released at a site >1 km away from the nearest source population, making it unlikely that females were counted twice. Populations were visited in at least 3 out of the 4 years, and although in some years a population may only have been visited once, most populations were usually sampled repeatedly over the season [mean number of visits per season (±SE): 3.41 ± 0.31, mean number of females caught per population each season: 39.77 ± 4.15]. The mean number of days between separate population visits was 9.65 ± 0.82 ($n = 139$ unique visits in total). As damselflies have high mortality and rarely survive more than a week in the wild (Cooper *et al.*, 1996; Corbet, 1999), the capture dates of individual damselflies were accordingly used as estimates of individual emergence dates.

Development time in laboratory-raised families

Female *I. elegans* of all three morphs (>25 full-sib families of each morph) were captured in the field and transported to our laboratory. Eggs were obtained by placing the females in small plastic cups with damp filter paper at the bottom. All females were from the same population (Vombs Vattenverk) except for a few *Infuscans-obsoleta* females, which are the rarest morph. Some *Infuscans-obsoleta* females were collected from other nearby populations in order to provide a balanced data set. Water was added to the egg-laying containers and the female removed after the eggs were laid. Once the eggs hatched, the larvae were transferred to large plastic containers and fed with brine shrimp (artemia) daily. Larvae were transferred to individual enclosures within the plastic containers after approximately 1 month, in order to prevent cannibalism. They were kept under a constant temperature and light regime (temperature: 17 °C, light regime: 12:12). Larvae were maintained in the lab until emergence next spring, after which females were released into insectaries and maintained on *Drosophila* until their morph status could be determined.

Statistics

Data were analysed using mixed models [PROC MIXED, SAS (Littell *et al.*, 1996)]. Development in the lab was analysed with maternal morph, sex and individual morph as fixed factors, whereas family was considered a random factor nested within maternal morph. Family was included in order to control for the nonindependence of emergence date of siblings (Fry, 1992) and was nested within maternal morph as each family can by definition only have one value of maternal morph (Littell *et al.*, 1996). Maternal morph and sex were included together in the analysis of all offspring (males and females), whereas maternal morph and individual morph were included in the analysis of female offspring. Interaction terms between fixed factors were included in both analyses. We could not include all three fixed factors (maternal morph, individual morph and sex) in one analysis because males are monomorphic. Interactions between random and fixed factors [family(maternal morph)*sex and family(maternal morph)*individual morph] were included in the model (Newman *et al.*, 1997) but because the interactions were nonsignificant and did not change the results only the reduced model is presented here.

For emergence in the field, a mixed model was used with morph as a fixed effect, and year and population as random effects. This is because both effects represent only a subsample of all potential years and populations (Fry, 1992). All interactions were initially included, but nonsignificant effects ($P > 0.05$) were sequentially removed from the final model, starting with the highest order interactions, and only the final, reduced model is presented here. In the analysis of both lab and field data, *post hoc* comparisons of least square means were performed.

Results

Emergence dates in the field

There was a significant effect of morph on capture date in the field, as well as significant effects of population and the population*year interaction (Table 1). *Infuscans* females emerged significantly later than both Androchrome ($P < 0.001$) and *Infuscans-obsoleta* females ($P < 0.05$; Fig. 1). There was no significant difference between the emergence dates of Androchromes and *Infuscans-obsoleta* females. The morph*year and morph*population interactions were not significant, which indicate that the general pattern of morph emergence was the same in all populations over all years, but that the actual emergence dates were earlier or later depending on the population and the year.

Table 1 Table of effects of morph, population, and year on capture date of field-caught females ($n = 2127$). Data were analysed using a mixed model with population and year as random effects, and morph as a fixed effect. For fixed effects (morph), the test statistic is *F*, for random effects (population, year, and population*year), it is *Z*. The initial model included all interactions, and nonsignificant interaction effects were sequentially removed (starting with the highest-order interactions) to give the final model presented here.

Effect	d.f.	*F*	*Z*	*P*-value
Morph	2	5.93		0.0027
Population	13		1.82	0.0346
Population*year	39		3.85	<0.0001

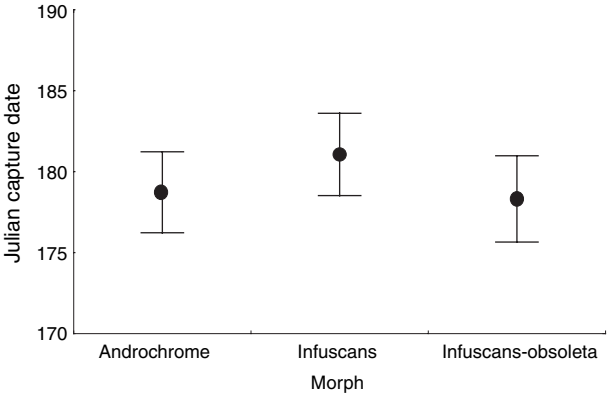

Fig. 1 Capture date (Julian day ± SE) in the field of females of the three morphs. *Infuscans* females were captured significantly later than either of the two other morphs ($P < 0.05$).

Development time of families and morphs in the laboratory

There were significant effects of sex, maternal morph and family on development time, but no effect of individual morph (Table 2). Males emerged earlier than females

Table 2 Table of effects of maternal morph, sex, individual morph, and family on development time in the laboratory. Maternal morph and sex were included in the first analysis (all offspring), maternal morph and individual morph in the second (females only) and maternal morph in the third (males only). All three analyses were mixed models with family as a random effect. For fixed effects (maternal morph, sex, individual morph), the test statistic is *F*; for random effects (family), it is Z.

Effect	d.f.	*F*	*Z*	*P*-value
All offspring ($n = 608$)				
Maternal morph	2	7.97		0.0007
Sex	1	12.77		0.0004
Maternal morph*sex	2	0.84		0.4342
Family(maternal morph)	77		4.33	<0.0001
Female offspring only ($n = 237$)				
Maternal morph	2	4.28		0.0175
Individual morph	2	0.43		0.6481
Maternal morph*individual morph	4	0.40		0.8064
Family (maternal morph)	74		3.45	0.0003
Male offspring only ($n = 342$)				
Maternal morph	2	7.27		0.0013
Family (maternal morph)	77		2.60	0.0047

(Fig. 2) and the offspring of *Infuscans-obsoleta* females emerged significantly earlier than the offspring of Androchrome ($P < 0.0001$) and *Infuscans* females ($P < 0.01$; Fig. 3). There was no significant difference between the offspring of Androchrome and *Infuscans* females. The effect of maternal morph was significant both when all individuals were included in the same analysis and when the sexes were analysed separately (males: $F_{2,342} = 7.27$, $P < 0.001$, females: $F_{2,266} = 6.04$, $P < 0.01$). Restricting the analysis to only Androchromes among the female offspring, we found that maternal morph also affected development time in this genetically more homogenous group ($F_{2,65} = 5.29$, $P < 0.01$), with a similar ordering between the family groups of the different maternal families (*Infuscans-obsoleta*: 285.02 ± 4.27; *Infuscans*: 298.48 ± 4.28; Androchrome: 302.18 ± 3.20).

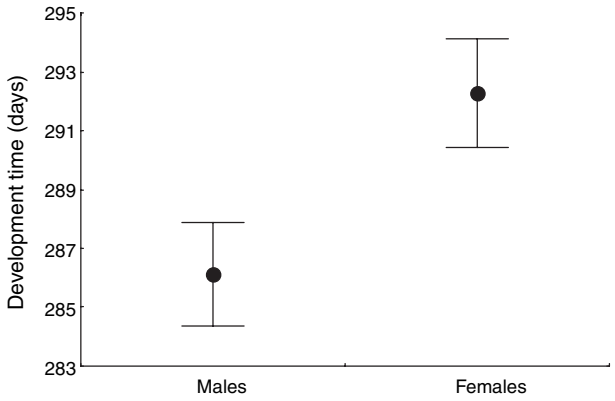

Fig. 2 Development time (days ± SE) in the laboratory in relation to sex. Males had a significantly shorter development time than females ($P < 0.001$).

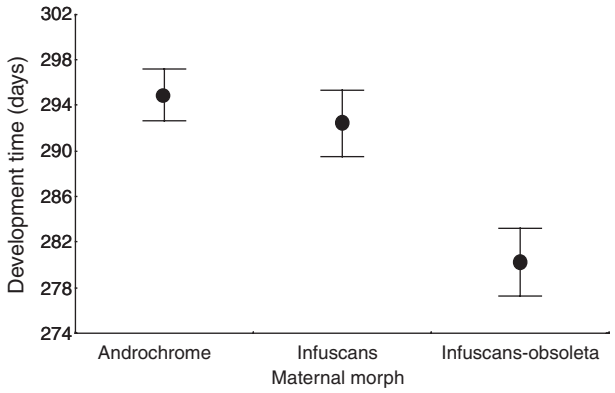

Fig. 3 Development time (days ± SE) in the lab for offspring of the three female morphs. Offspring of *infuscans-obsoleta* females had a significantly shorter development time than the offspring of the other two morphs ($P < 0.01$).

Discussion

Differences between morphs

Field and laboratory results in this study are concordant, revealing similar patterns of development time and emergence (Figs 1 and 3). The families of the different morphs emerged at different times in the laboratory, with *Infuscans-obsoleta* families emerging first and the families of the other two morphs later. This difference arose because larvae from *Infuscans-obsoleta* females grow faster (J. Abbott & E. I. Svensson, unpublished) and consequently emerged earlier (Fig. 3), though at the same size as the offspring of the other morphs (J. Abbott & E. I. Svensson, unpublished). *Infuscans-obsoleta* families always emerged the earliest, and there were consistent differences in emergence times between *Infuscans* and *Infuscans-obsoleta* morphs in both the lab and the field (Figs 1 and 3). Androchrome females emerged early in the field, but offspring of Androchrome females emerged late in the lab. The reason for this discrepancy is unknown, but could be the result of increased sensitivity to lab conditions in Androchromes (e.g. higher temperatures in the laboratory compared with natural ponds). The female morphs of *I. elegans* and other related polymorphic species have typically been considered to be pure colour morphs (Van Gossum *et al.*, 1999; Sirot & Brockmann, 2001; Andrés *et al.*, 2002), with the morphs being identical in most respects apart from colour and patterning. These new findings of differences between morphs in both development and emergence time, as well as fecundity differences (Svensson *et al.*, 2005) and differences in size, shape and growth rates (J. Abbott & E. I. Svensson, unpublished) provide the first evidence that other traits are phenotypically or genetically correlated with these colour differences.

The significant effect of maternal colour morph on offspring development time in both sexes (Table 2) is likely to reflect a genetic correlation between the maternal and offspring traits. As a caveat, we note that we cannot entirely exclude the possibility that this relationship could partly be influenced by early environmental effects or nongenetic maternal effects (Lynch & Walsh, 1998), e.g. different allocation of resources to the eggs provided by the three different colour morphs. Although it has previously been suggested that there could be differences between colour morphs at the larval stage in *I. elegans* (Cordero *et al.*, 1998), investigations have been hampered by the fact that morphs are not detectable at this stage in the life-cycle. In addition, sex-limited expression of colour in this system makes it impossible to assign males to the different morphs. Our study, which uses an experimental approach similar to another recent study on a polymorphic insect (Ahnesjö & Forsman, 2003), was partly inspired by the previous workers who

have tested indicator models in sexual selection by correlating paternal colouration traits with various measures of offspring condition or performance (Sheldon *et al.*, 1997, 2003). The advantage of using maternal values in our study is that we could include all individuals in the analysis of the laboratory data, including males, larvae and immature females, which do not express the colour patterns visible only among the adult female morphs.

Mechanistic basis of the correlation between morph and development time

The genetic correlation between traits (maternal morph and larval development time) seen here could either be caused by pleiotropic effects of single loci or linkage disequilibrium between loci (Lande, 1980, 1984). Though a direct pleiotropic effect of the morph locus is a possibility, we do not know at present which physiological pathways connect adult colour morph and larval development rate. However, differences between melanic and nonmelanic morphs have been found in many insect species (True, 2003), probably resulting from pleiotropic effects of melanin (Wittkopp *et al.*, 2003).

The lack of any detectable effect of an individual's own morph might arise from small sample sizes and lack of statistical power, as not all females survived until their morph could be determined [which usually requires approximately 5 days (Cooper *et al.*, 1996)]. However, our results are also consistent with linkage disequilibrium caused by correlational selection in the larval stage (Brodie, 1992). A selective association built up in the field between emergence time and morph would be broken up through recombination in the laboratory where selection is presumably absent. The result of this would be an effect of morph in the field and of maternal morph on laboratory-raised females, but no effect of a laboratory-raised female's own morph.

Protandry and ontogenetic sexual conflict

Incomplete sex-limitation raises the prospects of an ontogenetic sexual conflict between male and female development times (Chippindale *et al.*, 2001; Rice & Chippindale, 2001). Males emerged earlier than females in the laboratory (Fig. 2), a process known as protandry that is likely to be adaptive in *I. elegans*, because its advantage in male–male scramble competition for females (Andersson, 1994). Males and females may have thus have different optimal emergence times, but could be prevented from reaching their phenotypic optima because of a correlated response to selection in the other sex (Rice & Chippindale, 2001). We are currently investigating whether incomplete sex-limitation is restricted to development time, or whether the other differences between female morphs are also partly or completely expressed in males.

Differences between populations

In our laboratory study, we found differences in emergence time between families that could not be attributed to the effects of sex or maternal morph (Table 2). The larvae in the laboratory were kept under identical constant conditions, and variation in development time therefore seems to have a genetic component, which is potentially important in determining individual fitness in the field. Selection for different emergence time may fluctuate according to weather conditions between years, with different genotypes (families or morphs) being favoured in different years. The absence of morph*population or morph*year interactions in our analysis of field-caught females (Table 1) suggests that the morph-specific pattern of emergence is fairly consistent across all populations and years, although the average emergence times differ between populations and years.

The significant population effect on emergence shows that some populations are consistently earlier or later. This could be because of environmental effects on water temperature and perhaps genetic differences between populations (Table 1). Morph frequencies are also known to vary between populations and within populations over different generations (E. I. Svensson & J. Abbott, unpublished), which are of particular interest in relation to the spatial and temporal differences in emergence time demonstrated in this study. As *infuscans-obsoleta* and Androchromes females emerged earlier (Fig. 1), variable weather patterns over the summer could influence morph frequencies over subsequent generations.

Relevance to maintenance of the polymorphism

Because female morph is correlated with development time, selection on the colour locus may not be restricted to the mating interactions among adults, the focus of most previous studies. Selection on other traits, such as date of emergence, could potentially also affect morph frequency dynamics. Recently, Reinhold (Reinhold, 2000) presented a model which suggests that fluctuating selection can maintain sex-limited polymorphisms because the sex that does not express the polymorphic trait acts as a shield protecting temporarily disfavoured alleles from selection. There is the potential for substantial survival selection on emergence date, and as development time differs between morphs in the field (Fig. 1; Table 1), has a clear genetic component, and appears to be genetically correlated with colour (Table 2), such selection could result in a correlated response in morph frequencies across generations.

Conclusions

Results in this study suggest that there is a genetic correlation between morph and development time, and by extension, with emergence date in the field. This

presents us with several interesting avenues of further research, such as differential sensitivity of the morphs to abiotic or biotic conditions, the relative importance of maternal effects in this system, pleiotropy vs. linkage disequilibrium as the cause of the genetic correlation, incomplete sex-limitation of other traits associated with female morph and the possibility of different optimal emergence times of males and females. Although there is other evidence of frequency-dependent selection at the adult stage in *I. elegans* (Svensson *et al.*, 2005), the contribution of correlated responses to selection on larval traits to the maintenance of the polymorphism will require continued investigation.

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