Ontogeny and Population Biology of a Sex-Limited Colour Polymorphism

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

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ABSTRACT

Though colour polymorphisms in adult organisms of many taxa have been demonstrated to be adaptive in the context of sexual selection or predation, genetic and/or phenotypic correlations between colour and other phenotypic traits expressed early in ontogeny could 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, there was a significant correlation between maternal morph and larval development time in both sexes. This suggests that the phenotypic correlation observed in field-caught females has a parallel in a genetic correlation between maternal colour and offspring development time. Maternal colour morph frequency may thus exhibit a correlated response to selection on larval emergence dates. The existence of a genetic correlation in male offspring implies incomplete sex-limitation which may result in ontogenetic sexual conflict between selection for early male emergence (protandry) and emergence times associated with maternal morph.

Keywords: damselflies, heterochrony, *Ischnura elegans*, linkage disequilibrium, melanin, ontogeny, pleiotropy, protandry, sex-limitation, sexual conflict

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 (Turelli *et al.*, 2001; Schemske & Bierzychudek, 2001), and insects (Mallet & Joron, 1999; Forsman & Appelqvist, 1999), and have become classical study systems among evolutionary biologists and ecologists. The standard explanations of the maintenance of two or more colour morphs in a population often focus on intra- or interspecific biotic processes such as predation or sexual selection. Organisms may have different colouration as an adaptation to different niches, with each morph being more cryptic in its preferred habitat as a way to avoid predation (Cain & Sheppard, 1954; Cook, 1998; Davison, 2002). Predators that form a search image and prey more heavily on the most common morph cause negative frequency-dependent selection, where rare morphs have an advantage (apostatic selection; (Allen, 1988; Weale *et al.*, 2000; Shigemiya, 2004). Polymorphisms in predators may result in negative frequency-dependence if prey recognize common predator morphs more easily, or as a result of selection for cryptic colouration in different environments (Shine *et al.*, 1998). Defensive mimicry may also promote the evolution of different colour morphs, for example the Batesian and Mullerian mimics found in several butterfly species (Mallet & Joron, 1999).

Sexual selection may cause negative frequency-dependent selection when the rarer morph has a reproductive advantage. This could be through the avoidance of costs of mating, as

has been suggested for the white morph in *Colias* butterflies (Nielsen & Watt, 2000). Alternatively, sexual selection can maintain morphs through intrasexual interactions, as in the side-blotched lizard *Uta stansburiana*, where each of the three male throat colour morphs has highest reproductive success when rare (Sinervo & Lively, 1996). Sex-limited polymorphisms are usually assumed to be maintained via sexual selection and male-female interactions.

Models of the maintenance of colour polymorphisms implicitly assume that colour is only subject to selection in the context of sexual selection or predation. It is, however, possible that there are additional phenotypic differences between morphs that are also under selection, e. g. physiological traits that are expressed during earlier parts of the life-cycle. For instance, Sewall Wright studied genetic drift in the wild by examining what he assumed was a neutral trait: floral colour in the annual flower plant *Linanthus* (Provine, 1986). Yet later studies revealed that floral colour was actually associated with differential reproductive success of the morphs dependent on the amount of rainfall in the spring (Turelli *et al.*, 2001; Schemske & Bierzychudek, 2001). Other examples of traits 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; Wilson *et al.*, 2001; Svensson *et al.*, 2001a; Svensson *et al.*, 2001b; Svensson *et al.*, 2002; True, 2003). When colour morphs are genetically correlated with other traits, as in the cases cited above, it is possible that selection on these other traits can 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; Wong *et al.*, 2003; Sirot *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. The results in this study suggest that female colour morphs are both phenotypically and genetically correlated with larval development time and emergence date. This raises the question of the relative importance of direct selection on adult colour *per se* versus indirect selection on the colour locus due to its effect on larval traits earlier in ontogeny.

METHODS

Study species

The blue-tailed damselfly, *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 a male mimic. The two other morphs are often grouped together as gynochrome morphs (gynochrome=”female-coloured”), since their colouration is brown or green and potentially more cryptic (Cordero *et al.*, 1998). The *infuscans* (I) morph has the same black patterning as males and androchromes, but the eighth abdominal segment is brown instead of blue and the thorax is olive green. The *infuscans-obsoleta* (IO) morph has similar black patterning on the abdomen, but lacks two of the stripes on the thorax (the humeral stripes) and retains only the central black stripe. Otherwise it is similar to *infuscans* in colour, but is more brownish (Askew, 1988). Though *infuscans-obsoleta* females can be identified from first emergence due to their unique pattern of black colouration, 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); Cordero, personal communication). 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). Females of a given morph are, overall, more likely to produce offspring of the same morph, although actual probabilities depend on allele and heterozygote frequencies in the population.

Emergence of females in the field

14 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 in order to determine morph frequencies. Populations were visited in at least three out of the four 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. Since damselflies have high mortality and rarely survive more than a week in the wild (Cooper *et al.*, 1996; Corbet, 1999), capture date of individual damselflies was used as an estimate of individual emergence date.

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 is the rarest morph. To obtain approximately equal numbers of females of each morph and hence a balanced data set, some *infuscans-obsoleta* females had to be gathered from other nearby populations. 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 one 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 was 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, while family was considered a random factor nested within maternal morph. This is to control for the non-independence of emergence date of siblings (Fry, 1992). Family was nested within maternal morph because 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. All three fixed factors could not be included in one analysis because males are monomorphic. Family(maternal morph)*sex and family(maternal morph)*individual morph interactions were included in the model (Newman *et al.*, 1997) but the interactions were non-significant and did not change the results, so for simplicity only the reduced model with no interactions between random and fixed factors 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 non-significant effects (P>0.05) were sequentially removed from the final model, starting with the highest order interactions, so 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=0.0009) and *infuscans-obsoleta* females (P=0.0207; Figure 1). There was no significant difference between the emergence dates of androchromes and *infuscans-obsoleta* females (P=0.6959). The morph*year and morph*population interactions were not significant, which indicates that the general pattern of 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.

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 (Figure 2) and the offspring of *infuscans-obsoleta* females emerged significantly earlier than the offspring of androchrome (P=0.0001) and *infuscans* females (P=0.0038; Figure 3). There was no significant difference between the offspring of androchrome and *infuscans* females (P=0.4982). 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).

DISCUSSION

Differences between morphs

Field and laboratory results in this study are concordant, revealing the same general pattern 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. That there was no significant effect of individual morph in the laboratory study is probably due a relatively small sample size, since it was not possible to maintain all females alive in the laboratory until their morph was could be determined (which usually requires approximately 5 days (Cooper *et al.*, 1996)). *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). 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 all respects other than their colour. Our findings of differences between morphs in both development and emergence time (this study), as well as fecundity differences (Svensson *et al*. in review) and differences in size, shape, and growth rates (J. Abbott & E. I. Svensson, unpublished data) 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 non-genetic maternal effects (Lynch & Walsh, 1998), e. g. different allocation of resources to the eggs provided by the three different colour morphs. However, several independent lines of evidence speak against such an interpretation. First, maternal effects typically manifest themselves early in development, and their relative importance usually declines with offspring age (Wade ,1998). In the case of these damselfly larvae, we have found that morphological differences in size and shape between the morphs typically become apparent only late in ontogeny, close to emergence, whereas initial differences are small (J. Abbott and E. Svensson, unpublished data), which is consistent with late-acting genetic differences and inconsistent with early environmental or maternal effects. Damselfly larvae go through several instars throughout their ontogeny, each one of which

should partly diminish or wipe out any remaining maternal effects (Corbet, 1999). Second, we have found no differences in egg size between the different colour morphs and no significant differences in early growth rates, which further speaks against the importance of maternal effects. We thus tentatively conclude that the correlation between maternal colour morph and development time does indeed reflect a genetic correlation that is only slightly, if at all, influenced by early environmental or maternal effects.

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. In our laboratory study, we found evidence for a correlation between maternal colour morph and an offspring trait (development time), an approach that was partly inspired by previous studies in which workers have tested indicator models in sexual selection by correlating male secondary sexual colouration traits with various measures of offspring condition or performance (Sheldon *et al.*, 1997; Sheldon *et al.*, 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

Which physiological mechanism(s) could cause the observed differences in development and emergence time related to female morph? Genetic correlations between traits could either be caused by pleiotropic effects of single loci or linkage disequilibrium between linked or unlinked loci (Lande, 1980; Lande, 1984). A direct pleiotropic effect of the morph locus is a possibility, although we do not know at present which physiological pathways could connect adult colour morph and larval development rate. It has been shown in other polymorphic insects that melanic morphs often differ from their non-melanic counterparts in several different traits (True, 2003). Differences in pigmentation are likely to be the result of differences in gene regulation (Wittkopp *et al.*, 2003), so this may also be true of the traits that are correlated with different pigmentation. The difference in larval development time observed in the laboratory arose because larvae from *infuscans-obsoleta* females grow faster (J. Abbott and E. I. Svensson, unpublished data) and consequently emerged earlier (Fig. 3), though at the same size as the offspring of the other morphs (J. Abbott and E. I. Svensson, unpublished data). This could affect adult morphology through a process of heterochrony at the intraspecific level (West-Eberhard, 2003) since one of the morphs develops faster than the others. Heterochrony may be a common process in morphological evolution, and it is a way of increasing differences between groups through relatively minor genetic change (Gould, 2002). Results in this study indicate that intraspecific heterochrony could have played a role in the initial morphological divergence of the three morphs in *I. elegans.*

Although a direct pleiotropic effect of the morph locus is one possible explanation for our results, the correlation between morph and developmental time could also be due to

linkage disequilibrium between the morph locus and other loci affecting development time. Linkage disequilibrium could arise either through physical linkage between the morph locus and other loci, or through a process of correlational selection that would create a statistical association between linked or unlinked loci, in a balance against the eroding force of recombination (Lande, 1984; Sinervo & Svensson, 2002). The significant correlation of maternal morph on male developmental time in the laboratory is particularly interesting in this context. Males are monomorphic with respect to colour, and the colour locus is therefore sex-limited in its expression (Cordero, 1990). If sex-limited expression is complete, linkage disequilibrium between the morph locus and a locus affecting growth rate is a more likely explanation of the effects on males, since an unexpressed locus cannot by definition have pleiotropic effects. Alternatively, pleiotropy is still a possible explanation if the morph locus is expressed in males for development time, but unexpressed for male colouration. In either case (pleiotropy or linkage disequilibrium) the effect on male emergence is an example of incomplete sex-limitation, and illustrates how a female phenotypic trait can be partially expressed in males.

Protandry and ontogenetic sexual conflict

Incomplete sex-limitation raises the prospects of sexual conflict between male and female development times, through the process of "ontogenetic sexual conflict" (Chippindale *et al.*, 2001; Rice & Chippindale, 2001). Males and females may have different optimal emergence times, but each sex could be prevented from reaching its phenotypic optimum because of a correlated response to selection in the other sex that would move it away

from its optimum (Rice & Chippindale, 2001). More specifically, males emerged earlier than females in the laboratory, a process known as protandry (Fig. 2). Protandry can either be a direct result of selection for earlier emergence, or a by-product of selection for smaller size (Zijlstra *et al.*, 2002). There is evidence that smaller males have higher mating success (Cordero *et al.*, 1997; Carchini *et al.*, 2000), so early emergence may be the result of selection for smaller size. Alleles at some loci for early emergence time in males are then potentially in conflict with the morph locus, which also affects male emergence time (Fig. 2; Table 2). This would be an example of an inter-locus sexual conflict between loci for development time and the morph locus, both of which could affect male fitness through their effects on protandry. 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. Early emergence in the season could be favoured by natural selection, since it would allow earlier reproduction and therefore earlier hatching of eggs. Eggs that hatch earlier in the season would also have more time grow before winter sets in and which could lead to a competitive advantage for early-

hatched larvae. However, earlier in the season (i. e. June) the weather in southern Sweden is more unpredictable, and individuals emerging early may not have time to reach maturity and reproduce before being killed by a few days of colder temperatures and rain. Selection for different emergence time may therefore fluctuate between years, with different genotypes (families or morphs) being favoured in different years. In the analysis of field emergence dates, environmental heterogeneity is reflected in the significant population*year interaction (Table 1), which indicates that populations differ in their response to seasonal variation. A morph*population or morph*year interaction in this analysis would reveal that the pattern of emergence of the different morphs differed between populations and/or years. Because we found no evidence of such effects, we conclude that the morph-specific pattern of emergence is fairly consistent across all populations and years, but that 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 due to environmental effects on water temperature and perhaps genetic differences between populations, with the interaction effect corresponding to climatic differences between years (Table 1). Morph frequencies are also known to vary between populations and over time (Svensson & Abbott, in submission), which is of particular interest in relation to the spatial and temporal differences in emergence time demonstrated in this study. Since *infuscans-obsoleta* and androchromes females emerged earlier (Fig. 1), weather patterns over the summer could potentially influence morph frequencies in the next generation.

Conclusions

Our findings of correlations between female morph and development time indicate that selection on the colour locus may not be restricted to the density-dependent intersexual mating interactions among adults that have been the focus of most previous studies. This suggests that selection on other traits, such as date of emergence, may also affect morph frequency dynamics. Reinhold (Reinhold, 2000) recently presented a model of how fluctuating selection could maintain sex-limited polymorphisms which may be relevant here. The model suggests that fluctuating selection can be sufficient to explain the maintenance of sex-limited polymorphisms because the sex that does not express the polymorphic trait acts as a shield protecting temporarily unfavoured alleles from selection. There is the potential for substantial survival selection on emergence date, and since development time differs between morphs in the field (Fig. 1; Table 1), has a genetic component, and appears to be genetically correlated with maternal colour (Table 2), such selection could potentially result in a correlated response in morph frequencies across generations. In summary, variable weather conditions may result in fluctuating selection on emergence date, which may affect female morph frequencies as a side-effect, and males of *I. elegans* may act to prevent loss of morph alleles from such fluctuating selection. Fluctuating selection can be sufficient to maintain polymorphisms even if traits are not completely sex-limited, as in the case here for male emergence time, as long as the strength of selection is unequal between the sexes (Reinhold, 2000). Although we have evidence for a role of frequency-dependent selection at the adult stage which seems

to be important in the maintenance of the morphs in *I. elegans* (Svensson *et al*., in review), according to the findings in this study selection on correlated traits during the larval stage may also play an important role in this species.

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Table 1: Table of effects of morph, population, and year on capture date of field-caught females. 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 non-significant interaction effects were sequentially removed (starting with the highest-order interactions) to give the final model presented here.

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

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.

All offspring (N = 608)				
Effect	df	F	Z	*P*-value
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

Figure 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 ($P<0.05$).

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

Figure 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 ($P<0.01$).

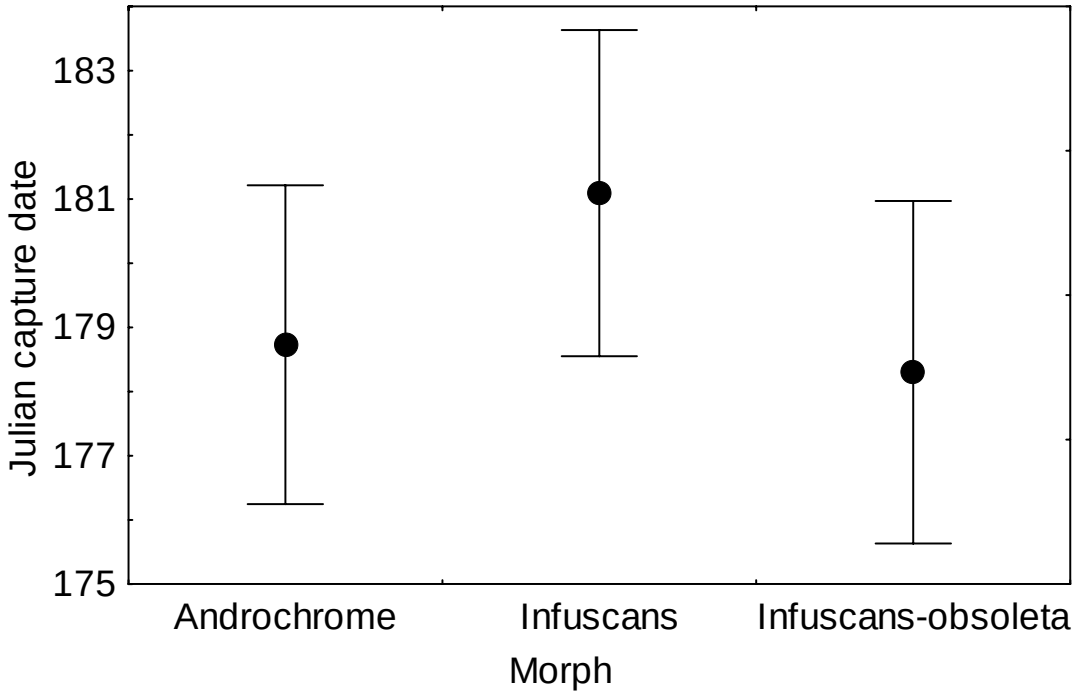
183
181
179
177
175
Julian capture date
Androchrome
Infuscans
Infuscans-obsoleta
Morph

Figure 1

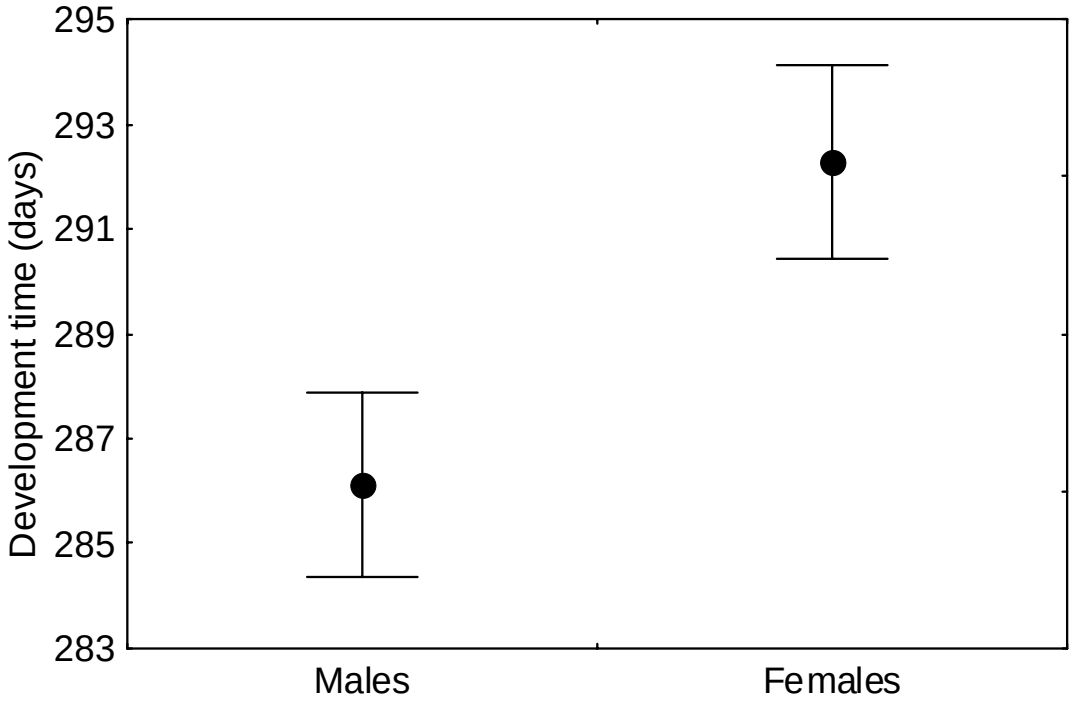
295
293
291
289
287
285
283
Development time (days)
Males
Females

Figure 2

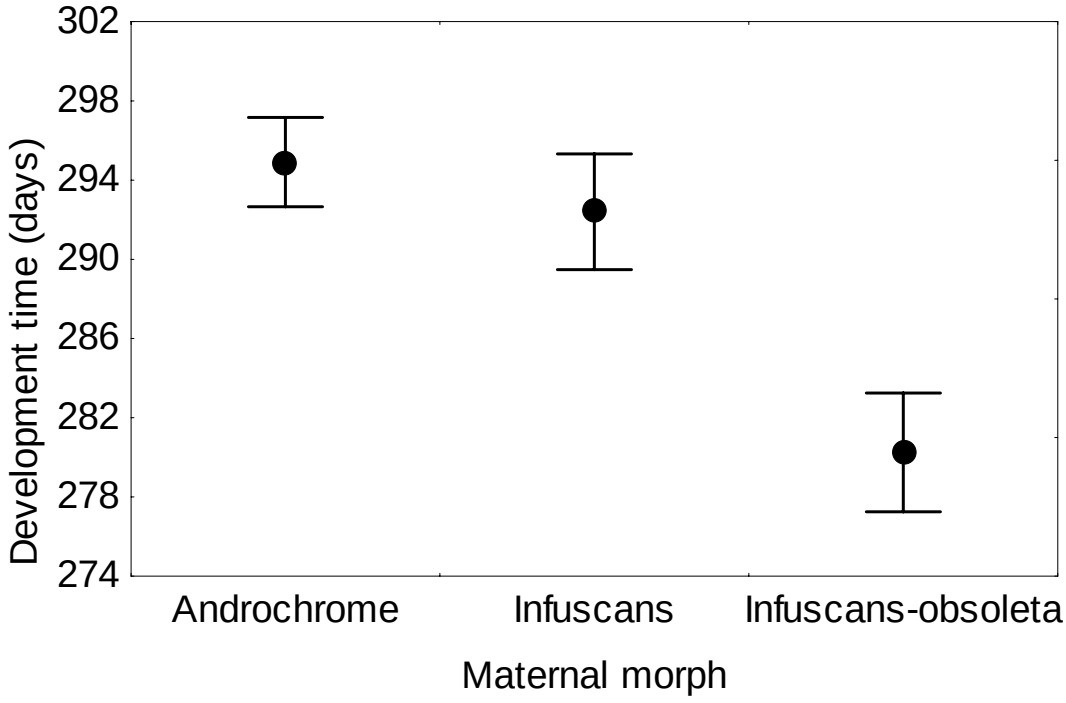
302
298
294
290
286
282
278
274
Development time (days)
Androchrome
Infuscans
Infuscans-obsoleta
Maternal morph

Figure 3