# Correlated morphological and colour differences among females of the damselfly *Ischnura elegans*

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ABSTRACT

1. The female-limited colour polymorphic damselfly *Ischnura elegans* has proven to be an interesting study organism both as an example of female sexual polymorphism, and in the context of the evolution of colour polymorphism. The study of colour polymorphism can also have broader applications as a model of speciation processes.

2. Previous research suggests that there exist correlations between colour morph and other phenotypic traits, and that the different female morphs in *I. elegans* may be pursuing alternative phenotypically integrated strategies. However, previous research on morphological differences in southern Swedish individuals of this species was only carried out on laboratory-raised offspring from a single population, leaving open the question of how widespread such differences are.

3. We therefore analysed multi-generational data from 12 populations, investigating morphological differences between the female morphs in the field, differences in the pattern of phenotypic integration between morphs, and quantified selection on morphological traits.

4. We found that consistent morphological differences did indeed exist between the morphs across all study populations, confirming that the previously observed differences were not simply a laboratory artefact. We also found, somewhat surprisingly, that despite the existence of sexual dimorphism in body size and shape, patterns of phenotypic integration differed most between the morphs and not between the sexes. Finally, linear selection gradients showed that female morphology affected fecundity differently between the morphs.

5. We discuss the relevance of these results to the male mimicry hypothesis and to the existence of potential ecological differences between the morphs.

## INTRODUCTION

Adaptation to different ecological conditions is well-recognized as both a potential route to speciation (Schluter, 2000; Nosil *et al.*, 2003; Vines & Schluter, 2006) and as the driver of the evolution of polymorphism (Galeotti *et al.*, 2003; Leimar, 2005; Ahnesjö & Forsman, 2006). Although ecological polymorphism is better studied to date, interest in sexual polymorphisms, particularly female-limited sexual polymorphisms, is on the rise (reviewed in Svensson *et al.*, in press). A recent review also highlighted the importance of studies of colour polymorphisms as model systems of speciation processes (Gray & McKinnon, 2007). An association between differences in colour and differences in other traits seems to be a common feature in colour polymorphic systems, and implies the existence of pleiotropic effects of colour on other traits such as morphology or behaviour. For example, both male and female colour morphs in the side-blotched lizard *Uta stansburiana* differ in aggression levels and in immune function (Svensson *et al.*, 2001; Mills *et al.*, 2008). Similarly, colour morphs of the grasshopper *Tetrix undulata* differ in body size even when reared under identical environmental conditions (Ahnesjö & Forsman, 2003).

The colour polymorphic damselfly *Ischnura elegans* has proven to be a useful study species both in the context of colour polymorphisms in general and of specifically female-limited sexual polymorphisms. The polymorphism in this species appears to be maintained, in part, by negative frequency-dependent selection (Svensson *et al.*, 2005) mediated by male mating harassment (Gosden & Svensson, 2007), and to be related to differences in morphology (Abbott & Svensson, 2008), development time (Abbott & Svensson, 2005), and patterns of intersexual genetic correlations (Abbott and Svensson, submitted), at least in the southern Swedish populations studied in these papers. There also appear to be differences in behaviour

between the morphs (Van Gossum *et al.*, 2001a). An interesting twist to this story is the fact that one of the female morphs is considered a male mimic (Robertson, 1985; Hinnekint, 1987; Svensson *et al.*, in press), and there is evidence both avoidance of male mimics by males (Cordero *et al.*, 1998; Hammers & Van Gossum, 2008) which appears to be density-dependent (Gosden and Svensson, submitted), and of learned mate recognition of common morphs (Van Gossum *et al.*, 2001b).

Although previous research has suggested that the female morphs in *Ischnura elegans* differ in morphology (Abbott & Svensson, 2008; Abbott and Svensson, submitted), these studies were based on laboratory-raised individuals from a single population. We were also interested in investigating whether male mimicry could affect selection on morphology and patterns of phenotypic integration between female morphs. Here, we present results from a more extensive analysis of multi-generational data from 12 populations, investigating morphological differences between the morphs in the field. We found that consistent morphological differences did indeed exist between the morphs across populations, that morph-specific patterns of phenotypic integration existed between traits, and that fecundity selection on these morphological traits differed between the morphs. We discuss the relevance of these results to the male mimicry hypothesis and to potential ecological differences between the morphs.

## METHODS

### Study species

The blue-tailed damselfly, *Ischnura elegans*, is a small species with three female morphs and monomorphic males (Corbet, 1999). *I. elegans* can be found in ponds set in open landscapes across Europe from southern Sweden to northern Spain. This species is univoltine in Sweden, although southern European populations are typically multivoltine (Askew, 1988). One of the morphs, the Androchrome (A), has similar blue colouration and black melanin patterning as males, and is considered a male mimic (Robertson, 1985; Hinnekint, 1987; Svensson *et al.*, in press). The Infuscans (I) morph is generally olive green when mature, but has the same black melanin patterning as males and Androchromes. The third morph, Infuscans-obsoleta (O), is olive green to brown when mature and generally has less black colouration the other morphs, including red (when immature) or brown (when mature) humeral stripes on the sides of the thorax rather than black humeral stripes (for photographs and illustrations see Svensson *et al.*, in press).

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 Androchrome allele (A) dominant to the Infuscans (I) and Infuscans-obsoleta (O) alleles and the I-allele dominant to the O-allele (i.e. A > I > O, Sánchez-Guillén *et al.*, 2005).

Data collection

We visited 12 populations outside Lund, in southern Sweden (Flyinge 30A1, Flyinge 30A3, Genarp, Gunnesbo, Habo, Hofterupssjön, Höje å 14, Höje å 6, Höje å 7, Lomma, Vallby mosse, and Vombs vattenverk) in the years 2002 to 2005. The geographic distance between these populations ranges from 1.08 to 41.11 km (mean = 14.54km). Our previous work

examining molecular population differentiation using AFLP-markers has shown no evidence of isolation by distance among these populations (Abbott, 2006). The average pairwise degree of genetic differentiation ($F_{st}$ ) between these populations is low to moderate and varies between 0.016 and 0.051 (Abbott *et al.*, 2008), indicating that these populations have diverged genetically but are not completely independent. Several of these populations have been relatively recently founded as part of a conservation program (Svensson & Abbott, 2005) and are subject to frequent population extinctions and recolonizations (E. I. Svensson, personal communication). These two factors possibly explain the observed increase in the degree of neutral molecular population differentiation over the course of only two generations (Abbott *et al.*, 2008). These aspects of the genetic population structure of our study populations suggest that these populations may not yet have reached their evolutionary equilibria.

In each population damselflies were regularly collected over each season and five different morphological measurements taken to the nearest 0.01 mm: total length, abdomen length, thorax width, wing length, and width of the fourth segment of the abdomen (S4). Significant narrow-sense heritabilities based on parent-offspring data have been found in four out of these five traits (mean $h^2$ forewing length: 0.463, total body length: 0.346, abdomen length: 0.242, thorax width: 0.173) when individuals have been raised in a common laboratory environment (Abbott, 2006). The genetic correlations between the traits are positive in all cases (range: 0.025 - 1) and are significant in 8 of the 10 cases (Abbott, 2006). A total of 4937 individuals are included in the analysis of morphology, 2741 males and 2196 females (1457 Androchromes, 563 Infuscans, and 176 Infuscans-obsoleta).

Fecundity data was collected as part of a long-term longitudinal investigation of our study populations (Svensson *et al.*, 2005; Svensson & Abbott, 2005; Gosden & Svensson, 2007; Gosden & Svensson, 2008; Gosden and Svensson, submitted). Field-caught females found in copula were set up in plastic oviposition chambers in an indoor laboratory and left for two days before being released. Eggs were counted on the third day. Sample sizes for the fecundity data were as follows: 953 Androchromes, 515 Infuscans, and 129 Infuscans-obsoleta. Our fecundity estimate is only a component of the total female lifetime fecundity, and as such may or may not reflect actual differences in lifetime reproductive success. However, it is known that fecundity from a single clutch can comprise 10-50% of the life-time fecundity in female damselflies (Fincke, 1986; Banks & Thompson, 1987; Corbet, 1999), and that inter-clutch intervals can be as short as one day (Banks & Thompson, 1987). A laying period of two days may therefore actually represent two clutches and is potentially a good measure of fitness, especially since there is no evidence of morph-specific differences in lifespan in this or in a closely related polymorphic species (Cordero, 1992; Cordero *et al.*, 1998; Andrés & Cordero Rivera, 2001). Our estimate is also likely to be a good fitness component given that female damselflies will lay a large proportion of the eggs present in the ovaries when presented with a favourable environment and left undisturbed (Corbet, 1999), which is the case here.

Analysis

All analyses were carried out in STATISTICA (Statsoft, 2004). We first looked for evidence of morphological differences between the sexes by carrying out a mixed-model MANOVA with all 5 morphological measures as dependent variables, and Year (random effect), Population (random effect), and Sex (fixed effect) as predictor variables (Population and Year

were random effects since our dataset represents a subsample of all possible years and populations, but the results do not change if they are instead treated as fixed effects). All two-way interactions were included in the model. We also carried out an analysis of morphological differences between the morphs using the same design, but with a fixed Morph effect in place of the Sex effect (we could not include both Sex and Morph in the same analysis since males are monomorphic). There was evidence of highly significant main effects of both Sex and Morph (see Results), confirming our expectation of the existence of morphological differences between these groups. In order to make these differences more readily interpretable in terms of overall size and shape and to avoid any problems associated with multicollinearity, we therefore performed a principal components analysis on all five morphological measurements, and selected the first two PCs for further analysis using mixed models of the same design as above.

Number of eggs laid was used in the calculation of linear selection gradients on all 5 morphological measures (Lande & Arnold, 1983). Selection analysis was carried out in several steps. First, morphological measures were standardized by female morph to a mean of zero and standard deviation of 1 within each morph. Second, relative fecundity was calculated separately for each morph. Standardized selection gradients were then estimated separately for each morph using mixed models with fecundity values as the dependent variable, Year and Population (and their interaction) as random effects to control for inter-population and inter-year differences in fecundity, and each trait as fixed continuous factors. We then tested for significant differences in the magnitude and/or direction of selection using a mixed model with Year and Population (and their interaction) as random factors, each trait as fixed continuous factors, and morph*trait interactions for each trait. In this analysis significant trait effects indicate significant linear selection on that trait which is consistent across morphs, and

significant trait*morph effects indicate that the magnitude and/or direction of selection on that trait is dependent on female morph. Note that we did not include a main effect of Morph in this analysis since fecundity values had already been standardized by female morph. Quadratic selection gradients were also investigated, but were found to be non-significant in all cases except one (there was some evidence of divergent selection on S4 width in Androchromes) and are therefore not presented. Similarly, we looked for evidence of variation in the strength and/or magnitude of selection between years and between populations (c.f. Gosden & Svensson, 2008) but found none (no significant year*trait or population*trait interactions) so results from this analysis are not presented either.

Conditional independence graphs were constructed after Magwene (2001). This method represents graphically the relationships between traits that remain after controlling for shared correlations between traits. This is done by calculating the phenotypic correlation matrix for the data set, inverting the matrix and then scaling the inverted matrix (Magwene, 2001), which results in a matrix of partial correlations for the dataset. The matrix of partial correlations is then tested for significance and strength of edges (Magwene, 2001) and presented graphically. These conditional independence graphs are a convenient way of visualizing phenotypic integration between traits (Magwene, 2001; Eroukhmanoff & Svensson, 2008). Similarity of phenotypic integration (partial correlation) matrices was analysed using mantel tests, and differences in the magnitude of correlations between groups were tested using t-tests. Although it would be interesting to see if differences in phenotypic integration patterns between the sexes and the morphs are also dependent on year and population, this would unfortunately result in very small sample sizes for some morph-year-population combinations, leading to unreliable partial correlation estimates. We have instead

198 elected to pool data from all years and populations and focus on general differences between
199 the sexes and the morphs.
200
201 RESULTS
202
203 Results from the MANOVA analyses indicated the existence of highly significant
204 morphological differences between the sexes ($F_{5, 4870} = 1424.3$, $P < 0.0001$) and the morphs
205 ($F_{10, 4228} = 11.0$, $P < 0.0001$). We therefore used PCA to obtain overall measures of size and
206 shape for further analysis. PC1 accounted for 63.98% of the total variation and was a measure
207 of overall size, since the factor loadings for all five traits were positive and large (Table 1).
208 PC2 accounted for 21.44% of the variation and had relatively high positive loadings on wing
209 length and abdomen width (S4) and high negative loadings on total length and abdomen
210 length (Table 1). This means that PC2 can be considered a measure of shape, and that
211 individuals with positive values of PC2 have relatively shorter, wider abdomens and longer
212 wings. This pattern of factor loadings for PC2 is qualitatively very similar to that found in a
213 previous laboratory analysis of morphology (Abbott & Svensson, 2008), and suggests that
214 results for shape differences are comparable between these studies. All subsequent PCs
215 accounted for approximately 8% of the variation or less, and were therefore not analysed any
216 further.
217
218 Analysis of PC1 (body size) showed that differences between populations varied between
219 years (significant Population*Year effect, Table 2). Females were larger than males in all
220 populations (significant effect of Sex, Table 2A, LS means ± SEs: females: 0.623 ± 0.020,
221 males: -0.656 ± 0.031), but the degree of sexual size dimorphism varied between populations
222 (significant effect of Population*Sex, Table 2A, Figure 1A) and years (significant effect of

223 Sex*Year, Table 2A, Figure 1B). Size differences between the female morphs trended toward
224 significance ($P < 0.08$ Morph effect, Table 2B, Figure 2A), and there was no evidence of
225 variation in size dimorphism between populations or years (no significant effects of
226 Population*Morph or Morph*Year, Table 2B), in contrast to results for sexual size
227 dimorphism. Post-hoc tests showed that Infuscans females were significantly larger than
228 Androchrome and Infuscans-obsoleta females (Fig 2A, all $P < 0.01$, LS means ± SEs:
229 Androchromes: 0.613 ± 0.027, Infuscans: 0.718 ± 0.046, Infuscans-obsoleta: 0.578 ± 0.070).
230
231 Differences in PC2 (body shape) between populations were also dependent on year
232 (significant Population*Year effect, Table 3). There was sexual dimorphism in body shape
233 (PC2) in all populations (significant effect of Sex, Table 3A), and the difference between the
234 sexes was greater in some populations than in others (significant Population*Sex effect, Table
235 3A, Figure 1C), but there was no effect of year on sexual dimorphism in shape (no effect of
236 Year*Sex, Table 3A). Males had lower values of PC2 than females, in other words longer,
237 narrower abdomens and shorter wings than females (LS means ± SEs: females: 0.711 ± 0.021,
238 males: -0.597 ± 0.033). The female morphs also differed in body shape (significant effect of
239 Morph, Table 3B). Androchromes had significantly more male-like morphology (i.e. longer,
240 narrower abdomen and shorter wings) than Infuscans and Infuscans-obsoleta females ($P <$
241 0.0001, Figure 2B, LS means ± SEs: Androchromes: 0.577 ± 0.029, Infuscans: 0.887 ± 0.049,
242 Infuscans-obsoleta: 0.867 ± 0.075). As with overall size differences, this pattern was constant
243 across populations (no significant effect of Population*Morph, Table 3B) and years (no
244 significant effect of Year*Morph, Table 3B).
245
246 Conditional independence analysis revealed a unique pattern of phenotypic integration in
247 Infuscans-obsoleta females (Figure 3). Mantel tests demonstrated that all phenotypic

integration (partial correlation) matrices were highly related, with correlation coefficients greater than 0.9 (males vs. Androchromes: r = 0.9798, males vs. Infuscans: r = 0.9640, males vs. Infuscans-obsoleta: r = 0.9192, Androchromes vs. Infuscans: r = 0.9825, Androchromes vs. Infuscans-obsoleta: r = 0.9306, and Infuscans vs. Infuscans-obsoleta: r = 0.9398; all $P <$ 0.0001). However, from these correlation coefficients we could see that correlations involving Infuscans-obsoleta were somewhat lower than correlations involving the other two morphs (0.91-0.94 and 0.96-0.99, respectively), and this difference is in fact significant when tested using a t-test (t = 5.49, df = 4, $P = 0.005$). This suggests that phenotypic integration patterns in Androchromes, Infuscans females, and males are all more closely related to each other than any of them are to Infuscans-obsoleta females. In contrast, correlations between the sexes are not lower than correlations within the sexes (i.e. between female morphs; t = 0.139, df = 4, $P = 0.896$), so there do not seem to be any large overall differences in phenotypic integration patterns between the sexes. From visual inspection of the phenotypic integration graphs, we can see that Androchromes and Infuscans females had very similar patterns of phenotypic integration, differing only in the strength of some of the partial correlations. Likewise, males had a very similar pattern of phenotypic integration to both Androchromes and Infuscans females, only differing in the addition of a new weak edge between abdomen length and thorax width. In contrast, Infuscans-obsoleta females not only lacked two of the edges present in other females, but also exhibited a unique edge between abdomen width (S4) and total length. This amounts to a 30% difference in presence/absence of edges (3/10 possible edges) between Infuscans-obsoleta and the other two morphs. The high partial correlations between total length and abdomen length seen in all groups are probably because these traits are not completely independent (abdomen length is a component of total length).

There was also evidence that morphological differences had morph-specific fitness consequences. Selection gradients on total length, abdomen length, abdomen width, and wing length differed significantly between the morphs (Table 4A). Androchrome females experienced significant positive selection S4 width, Infuscans females experienced significant negative selection on total length but positive selection on abdominal length, and Infuscans-obsoleta females experienced significant positive selection on S4 width but negative selection on wing length (Table 4B).

## DISCUSSION

Previous research on laboratory-raised individuals from a single population suggested that the female colour morphs in *Ischnura elegans* differed in morphology (Abbott & Svensson, 2008). In this study we found that morphological differences observed in the field were generally similar to those previously observed in the laboratory (Abbott & Svensson, 2008). This study therefore provides clear evidence that the existence of morphological differences between female colour morphs in *I. elegans* is not simply a laboratory artefact, nor the property of a single population, but is in fact a consistent feature both over time and across all 12 populations studied here.

Sexual size dimorphism is common in damselflies and in non-territorial species such as *I. elegans* females are usually larger than males (Corbet, 1999). Both this fact and previous results (Abbott & Svensson, 2008) led us to expect to find sexual dimorphism in body size and shape. Indeed, males were smaller than females, with relatively longer, narrower abdomens and shorter wings (Figure 1). Differences in body shape are likely to be related to the positions of the sexes during mating and fecundity selection in females, as discussed in

297 Abbott & Svensson (2008). Interestingly, the degree of sexual dimorphism in size and shape
298 varied between populations and years (Tables 2A and 3A). This could be a result of
299 differential sensitivity of the sexes to different abiotic or biotic environmental conditions
300 between populations (Badyaev, 2002). For example, it has previously been found that
301 photoperiod and temperature jointly affect the degree of SSD in the damselfly *Lestes viridis*
302 (de Block & Stoks, 2003). Similarly, spatial and temporal fluctuations in the strength of
303 fecundity selection in females or of sexual selection in males (Gosden & Svensson, 2008)
304 could also produce varying patterns of SSD. Finally, variation in morph frequencies between
305 years/populations in combination with overall size differences between the morphs (see
306 below) could also partly explain spatial and temporal variation in the degree of SSD. Because
307 Infuscans females are larger overall than the other morphs, populations/years with a high
308 frequency of Infuscans females could have higher SSD than populations/years with a low
309 frequency of this morph, assuming male size is more or less constant.
310
311 Though it has previously been found that Androchromes may be larger than the other morphs
312 in a closely related species (Cordero, 1992), this was not the case in our study populations.
313 Infuscans females were larger than the other morphs, and Androchrome females had
314 relatively longer, narrower abdomens and shorter wings than the other morphs (Figure 2).
315 These consistent morphological differences are particularly striking since they exist despite
316 clinal variation in body size along the coastal-inland gradient in these populations (Gosden &
317 Svensson, 2008), and stand in sharp contrast to the observed temporal and spatial variation in
318 the degree of sexual dimorphism. Female fecundity is often related to body size in insects
319 (Bonduriansky, 2001), and since previous results (Svensson & Abbott, 2005) indicate that
320 Infuscans females have higher overall fecundity than the other morphs, it seems reasonable
321 that this elevated fecundity is partially the result of their larger size. However we did not find

any evidence of selection for larger thorax width, which is the best predictor of overall size (i.e. highest loading on PC1; Table 1), and Infuscans females actually experienced negative selection on total body length (Table 4). This suggests that other selective pressures than fecundity selection may be influencing female size, which is rather surprising given widespread evidence of fecundity selection on size in insects (Bonduriansky, 2001). It is, however, consistent with previous work in two other damselfly species which have found that female size was not related to fecundity (Anholt, 1991; Richardson & Baker, 1997).

The difference in body shape between Androchromes and the other morphs is analogous to the differences between the sexes, though smaller in magnitude (see Results). One common explanation of the maintenance of the polymorphism in this and related species is that Androchrome females are male mimics, and therefore avoid costs of male mating harassment (e.g. Cordero *et al.*, 1998; Cordero Rivera & Sánchez-Guillén, 2008), and other studies have found evidence of phenotypic similarity of Androchromes to males in colouration and black patterning (Joop *et al.*, 2006; Van Gossum *et al.*, 2008). Although the male mimicry hypothesis only explicitly deals with similarity in colouration between males and Androchrome females, correlated morphological and colour differences in other polymorphic species from a range of taxa (see Introduction) suggest that morphological mimicry could also be a possibility. The more masculine phenotype typical of Androchromes is consistent with this explanation, although other frequency- and density-dependent factors are known to be at work in these populations (Svensson *et al.*, 2005; Gosden & Svensson, 2007). Some studies suggest that Androchromes are always less preferred by males than other morphs (Hammers & Van Gossum, 2008; Cordero Rivera & Sánchez-Guillén, 2008), while others suggest that males learn to recognize and prefer common morphs (Van Gossum *et al.*, 2001a; Van Gossum *et al.*, 2001b; Fincke *et al.*, 2007). Male mimicry and learned mate recognition need not be

mutually exclusive, however, for example if Androchromes must reach higher frequencies than other morphs before males learn to recognize them. Despite evidence of morphological male mimicry in Androchromes, we did not find any clear evidence of selection for masculinized morphology in Androchromes or, alternatively, against masculinized morphology in the other morphs. It is possible that Androchromes are already at or near their morphological optimum and only experience weak stabilizing selection on morphology. It is also possible that our fecundity estimates did not capture aspects of fitness that are subject to selection for masculinization, for example if more masculinized morphology in Androchromes affects survival. However, weak stabilizing selection is unlikely since we found no evidence of stabilizing selection for any trait in Androchromes (data not shown), and there is no evidence of differences in lifespan between morphs in a related polymorphic species (Andrés & Cordero Rivera, 2001), which speaks against effects of survival selection. This suggests that morphological similarity between males and Androchromes could be the result of pleiotropic effects at the morph locus rather than selection for masculinized morphology. Alternatively, Androchromes could suffer a trade-off between maximising their fecundity and minimising male mating harassment through male mimicry (Gosden and Svensson, submitted) resulting in no net selection for masculinized morphology.

Conditional independence analysis (Magwene, 2001) also revealed differing patterns of phenotypic integration between the morphs. Interestingly, rather than seeing a large difference in the pattern of phenotypic integration between the sexes, which is what one might expect based on the existence of sexual dimorphism in size and shape in *I. elegans* (see above), the largest difference in phenotypic integration was between Infuscans-obsoleta females and the other morphs (Figure 3). This is consistent with laboratory results on morphology (Abbott & Svensson, 2008) and development time (Abbott & Svensson, 2005), which also found that

Infuscans-obsoleta females were the most divergent morph. Why this large difference in the pattern of phenotypic integration between Infuscans and Infuscans-obsoleta females does not seem to be reflected in a large difference in PC2 (body shape) is unknown, but could simply be because PC2 is capturing other aspects of shape variation than the phenotypic integration analysis (Jackson, 1991). This is possible since PC2 is likely to be more heavily influenced by differences in shape between the sexes than by differences in shape between the morphs. One of the unique features of the pattern of phenotypic integration in Infuscans-obsoleta was the presence of an edge between abdominal width (S4) and total length. Furthermore, the strongest positive selection gradient in the selection analysis was on S4 width in Infuscans-obsoleta females ($\beta > 0.3$, Table 4). It is tempting to speculate that these two results are related, and that strong selection on abdominal width in Infuscans-obsoleta females has resulted in increased phenotypic integration of this trait compared to the other morphs. Similarly, the strongest negative selection gradient in the selection analysis was on wing length in Infuscans-obsoleta females ($\beta < -0.3$, Table 4), and Infuscans-obsoleta is the only group lacking significant integration between abdomen length and wing length. Perhaps strong negative selection on wing length in this morph has resulted in a decoupling of wing length and abdomen length. However *why* Infuscans-obsoleta females experience such strong selection on these particular traits is currently unknown. More research on differing patterns of phenotypic and genetic integration of traits between the morphs is obviously needed if a detailed understanding of their evolution is to be achieved.

If fecundity selection for increased size or for morphological male mimicry in Androchromes cannot explain the morph-specific patterns of selection on morphology seen here, another possibility could be that each morph is selected to be better adapted to slightly different ecological conditions. Morph frequencies in this species differ both between geographical

regions in Europe (Gosden, 2008) and between newly-established and older populations within southern Sweden (Svensson & Abbott, 2005), suggesting a role for ecological specialization and local adaptation in determining morph frequencies. Note that ecological differences between the morphs and the existence of negative frequency-dependence are not mutually exclusive. Ecological differences between the morphs could determine the range of morph frequencies that are stable in different populations or regions, while frequency-dependence could regulate morph frequency dynamics within that range (Andrés *et al.*, 2000; Abbott *et al.*, 2008). For example, ecological determination of stable ranges of morph frequencies have been found in the candy-strip spider *Enoplagnatha ovata* (Oxford, 2005). The existence of ecological differences between the morphs and their interaction with other factors is a potentially productive area for future research.

We have previously argued that the female morphs in *I. elegans* may be pursuing alternative phenotypically integrated strategies (Abbott & Svensson, 2008). The existence of correlated differences in morphological (this study), behavioural (Van Gossum *et al.*, 2001a; Gosden & Svensson, 2007), and life history traits (Abbott & Svensson, 2005; Svensson & Abbott, 2005) between morphs of *I. elegans* in our study populations support this idea, as does recent research showing differential effects of male mating harassment on the morphs (Gosden and Svensson, submitted). Although more research is needed before full knowledge of the nature of these strategies is achieved, this system has the potential to become a model system for the evolution of alternative female sexual polymorphisms (Svensson *et al.*, in press).

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Table 1: Factor loadings for the first and second principal components calculated from five morphological traits. PC1 is a measure of overall size and accounted for 63.98% of the total variation in morphology between individuals. PC2 is a measure of body shape, where individuals with positive values of PC2 have longer wings and wider but shorter abdomens, and accounted for 21.44% of the total variation in morphology between individuals.

| Measurement | Loading PC1 | Loading PC2 |
|---|---|---|
| Total length | 0.8234 | -0.4916 |
| Abdomen length | 0.7930 | -0.5397 |
| Thorax width | 0.8449 | 0.0925 |
| S4 width | 0.7086 | 0.6181 |
| Wing length | 0.8224 | 0.3850 |


28

Table 2: Results of statistical analysis of body size (PC1) using mixed models. Population and Year are random effects, as are all interactions with Population and Year. Sex and Morph are fixed effects, and were included in separate analyses (see Methods). N = 4937 (all individuals) for Sex (A), and N = 2196 (females only) for Morph (B).

| Effect | Df | MS | F | *P*-value |
|---|---|---|---|---|
| A) | | | | |
| Population | 11 | 46.18 | 20.14 | <0.0001 |
| Sex | 1 | 570.8 | 423.9 | <0.0001 |
| Year | 3 | 36.99 | 19.40 | 0.0001 |
| Population*Sex | 11 | 1.200 | 2.832 | 0.0011 |
| Population*Year | 33 | 2.108 | 4.975 | <0.0001 |
| Sex*Year | 3 | 2.004 | 4.730 | 0.0027 |
| Error | 4874 | 0.424 | | |
| B) | | | | |
| Population | 11 | 20.49 | 24.21 | <0.0001 |
| Morph | 2 | 1.221 | 3.116 | 0.0768 |
| Year | 3 | 11.92 | 17.04 | <0.0001 |
| Population*Morph | 22 | 0.448 | 0.990 | 0.4733 |
| Population*Year | 33 | 1.393 | 3.077 | <0.0001 |
| Morph*Year | 6 | 0.359 | 0.792 | 0.5761 |
| Error | 2118 | 0.453 | | |

Table 3: Results of statistical analysis of body shape (PC2) using mixed models. Population and Year are random effects, as are all interactions with Population and Year. Sex and Morph are fixed effects, and were included in separate analyses (see Methods). N = 4937 (all individuals) for Sex (A), and N = 2196 (females only) for Morph (B).

| Effect | Df | MS | F | *P*-value |
|---|---|---|---|---|
| A) | | | | |
| Population | 11 | 14.95 | 8.946 | <0.0001 |
| Sex | 1 | 596.6 | 860.7 | <0.0001 |
| Year | 3 | 13.29 | 15.57 | <0.0001 |
| Population*Sex | 11 | 1.131 | 2.310 | 0.0081 |
| Population*Year | 33 | 1.407 | 2.873 | <0.0001 |
| Sex*Year | 3 | 0.432 | 0.882 | 0.4498 |
| Error | 4874 | 0.490 | | |
| B) | | | | |
| Population | 11 | 4.971 | 6.676 | <0.0001 |
| Morph | 2 | 11.89 | 16.09 | 0.0005 |
| Year | 3 | 13.21 | 16.71 | <0.0001 |
| Population*Morph | 22 | 0.572 | 1.106 | 0.3318 |
| Population*Year | 33 | 0.986 | 1.906 | 0.0015 |
| Morph*Year | 6 | 0.824 | 1.594 | 0.1449 |
| Error | 2118 | 0.517 | | |

Table 4: Summary of results of selection gradient analysis for five morphological traits (significant values are highlighted in bold). A) Results of analysis to identify traits with morph-specific variation in the magnitude and/or direction of selection. There was evidence of variation in overall fecundity levels between years and populations, and of overall positive selection on S4 width and wing length. However, all traits except thorax width also showed evidence of morph-specific effects on the magnitude and/or direction of selection. B) Morph-specific selection gradients for all five morphological traits (SEs reported in brackets) calculated from separate analyses for each morph (see Methods). Androchrome females experienced significant positive selection on S4 width, Infuscans females experienced significant negative selection on total length but positive selection on abdominal length, and Infuscans-obsoleta females experienced significant positive selection on S4 width but negative selection on wing length.

A)

| Effect | Df | MS | F | *P*-value |
|---|---|---|---|---|
| Population | 11 | 1.864 | 1.692 | 0.1116 |
| **Year** | 3 | 2.943 | 3.323 | **0.0251** |
| **Year*Population** | 32 | 1.249 | 2.380 | **<0.0001** |
| Total length | 1 | 0.051 | 0.096 | 0.7563 |
| Abdomen length | 1 | 0.352 | 0.671 | 0.4130 |


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| | | | | |
|---|---|---|---|---|
| Thorax width | 1 | 0.004 | 0.007 | 0.9328 |
| **S4 width** | 1 | 8.885 | 16.94 | **<0.0001** |
| **Wing length** | 1 | 2.915 | 5.556 | **0.0185** |
| **Total length*Morph** | 2 | 1.623 | 3.093 | **0.0457** |
| **Abdomen length*Morph** | 2 | 1.943 | 3.703 | **0.0249** |
| Thorax width*Morph | 2 | 0.050 | 0.094 | 0.9100 |
| **S4 width*Morph** | 2 | 3.490 | 6.653 | **0.0013** |
| **Wing length*Morph** | 2 | 1.999 | 3.810 | **0.0224** |
| Error | 1535 | 0.525 | | |

B)

| Trait | Androchrome | Infuscans | Infuscans-obsoleta |
|---|---|---|---|
| Total length | 0.0838 (0.0476) | **-0.1576 (0.0761)** | 0.0478 (0.3467) |
| Abdomen length | -0.0521 (0.0514) | **0.1680 (0.0710)** | 0.1659 (0.3319) |
| Thorax width | -0.0003 (0.0371) | 0.0109 (0.0500) | -0.0646 (0.1427) |
| S4 width | **0.0918 (0.0306)** | -0.0023 (0.0411) | **0.3620 (0.1404)** |


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| | | | |
|---|---|---|---|
| Wing length | 0.0088 (0.0295) | 0.0007 (0.0472) | **-0.3739 (0.1805)** |


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Figure 1: Sexual dimorphism in body size (PC1) according to A) population, and B) year, and sexual dimorphism in C) body shape (PC2) according to population. Population abbreviations are as follows: F1 = Flyinge 30A1, F3 = Flyinge 30A3, Ge = Genarp, Gu = Gunnesbo, Ha = Habo, Hof = Hofterupssjön, H14 = Höje å 14, H6 = Höje å 6, H7 = Höje å 7, L = Lomma, VM = Vallby mosse, and Vo = Vombs vattenerk. Females are always significantly larger than males, but the degree of sexual size dimorphism varied between populations and years. Similarly, males have relatively longer, narrower abdomens and shorter wings than females (lower values of PC2) but the magnitude of differences in body shape between the sexes varied between populations. Error bars denote SEs. Note that cartoon damselflies are for illustrative purposes only and do not reflect the magnitude of actual differences between the sexes.

Figure 2: Differences in between the morphs in A) Body size (PC1). Infuscans females are the largest overall. B) Body shape (PC2). Androchromes are most male-like in shape, while Infuscans and Infuscans-obsoleta females are less male-like and very similar in shape. Error bars denote SEs. Note that cartoon damselflies are for illustrative purposes only and do not reflect the magnitude of actual differences between the morphs.

Figure 3: Phenotypic integration graphs for A) Males (N = 2741 individuals), B) Androchrome females (N = 1457 individuals), C) Infuscans females (N = 564 individuals), and D) Infuscans-obsoleta females (N = 176 individuals). Partial correlations which are significant at the 0.05 level are shown, and values are reported adjacent to lines between traits. Strong edges are indicated by heavy lines, weak edges by light lines. The high partial correlations between total length and abdomen length present in all groups are because these

traits are not completely independent. Note the unique pattern of phenotypic integration in Infuscans-obsoleta females.


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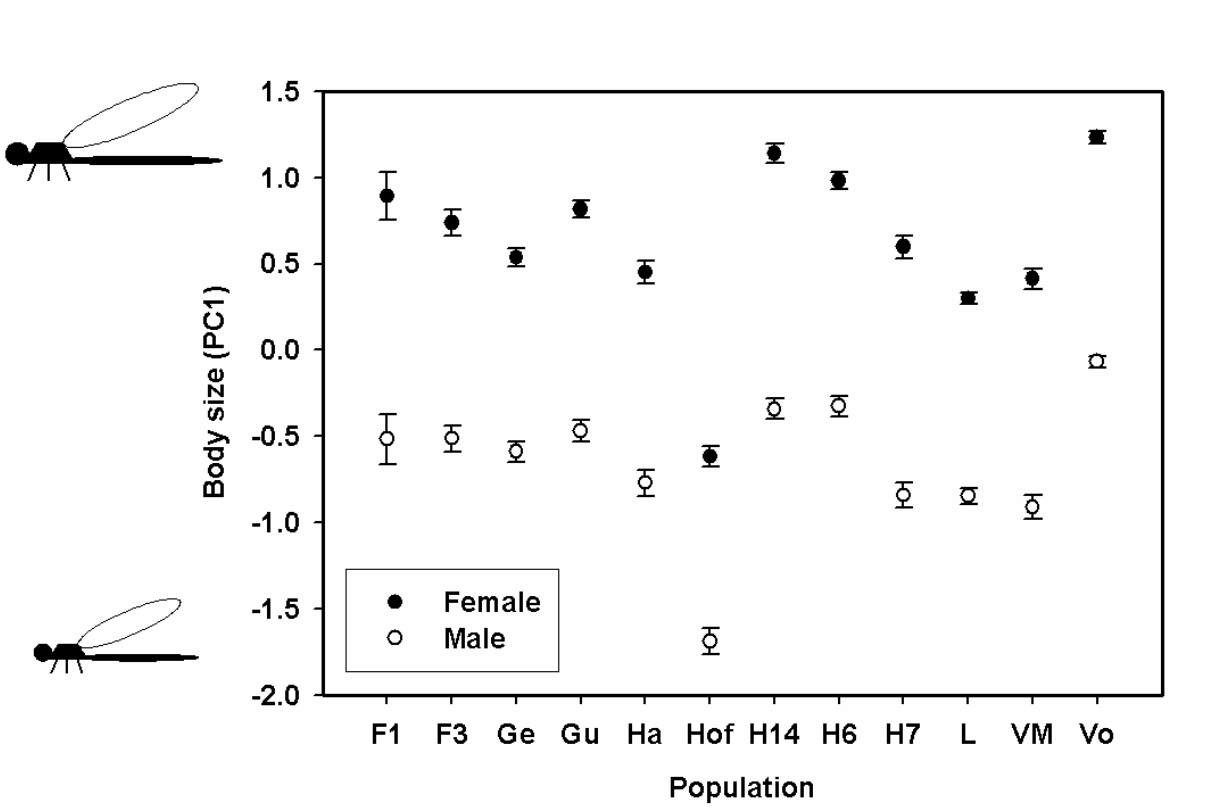
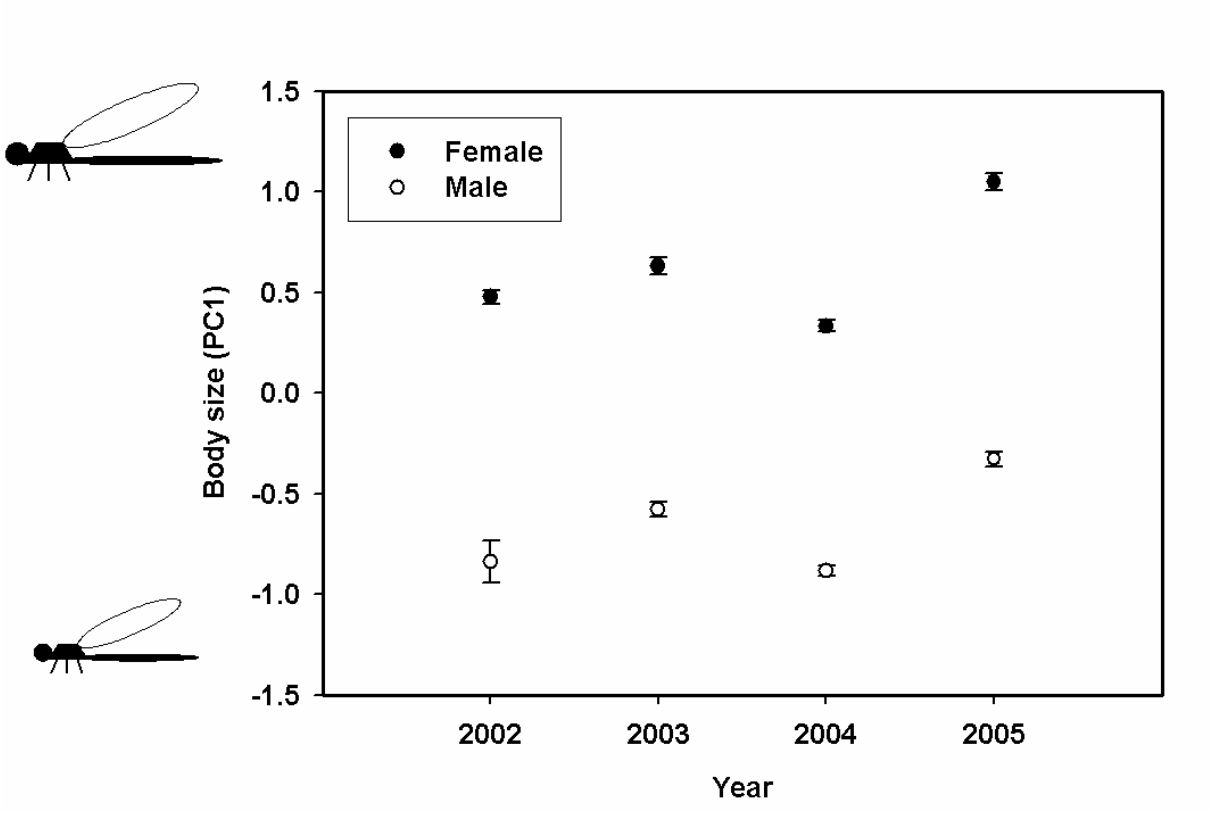



596 Figure 1A

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598 Figure 1B



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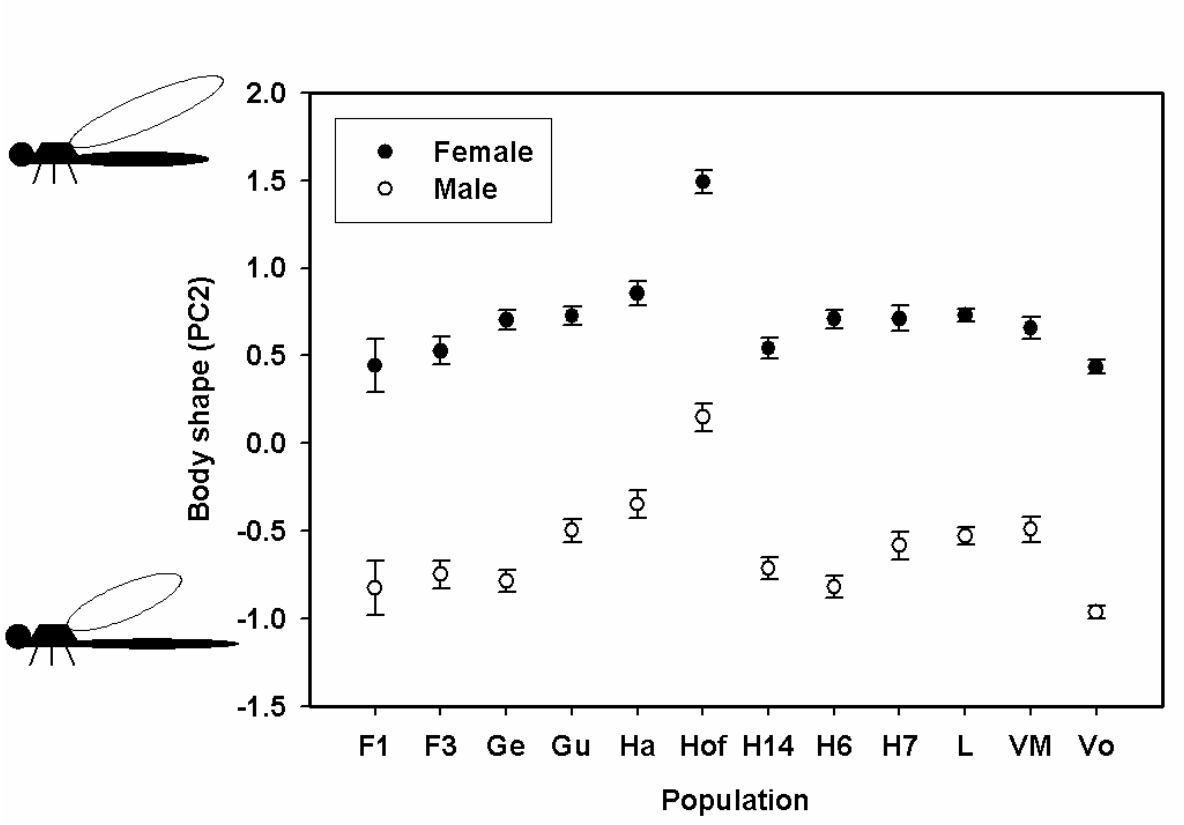


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600 Figure 1C



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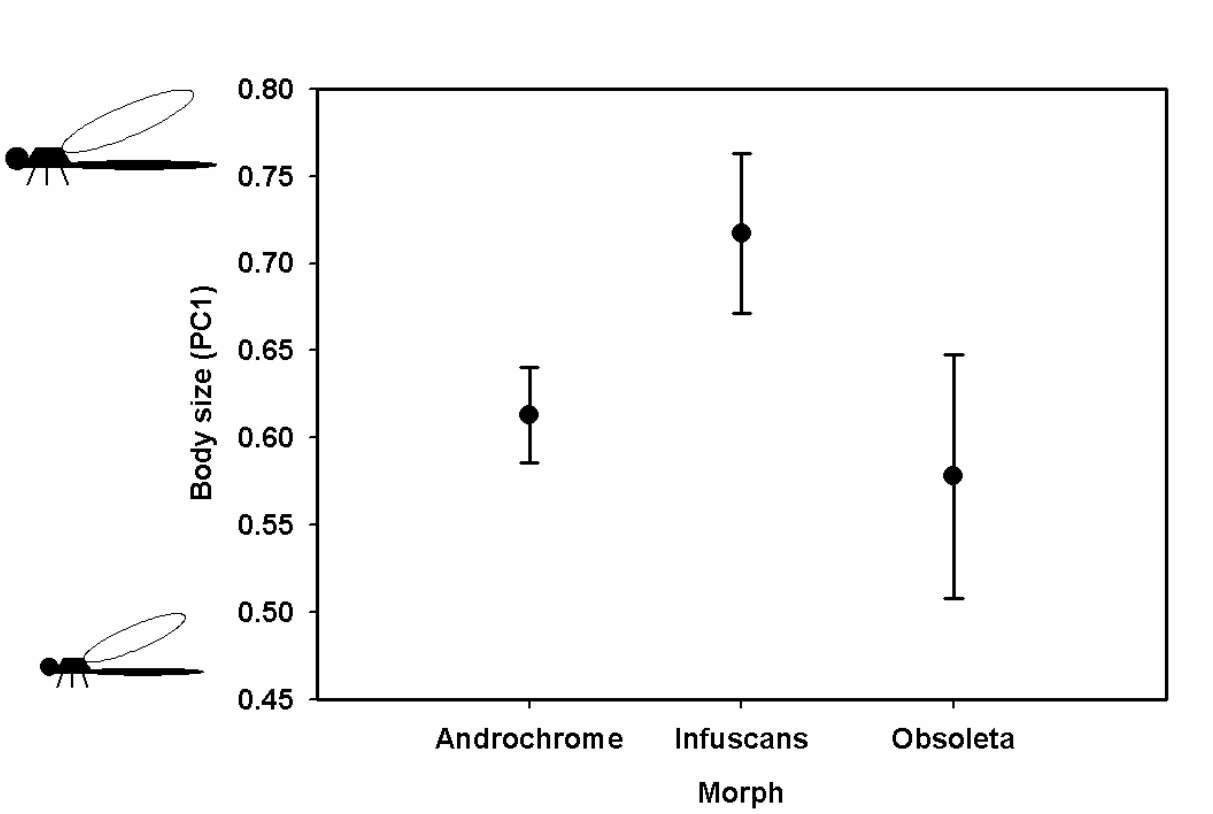


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602 Figure 2A



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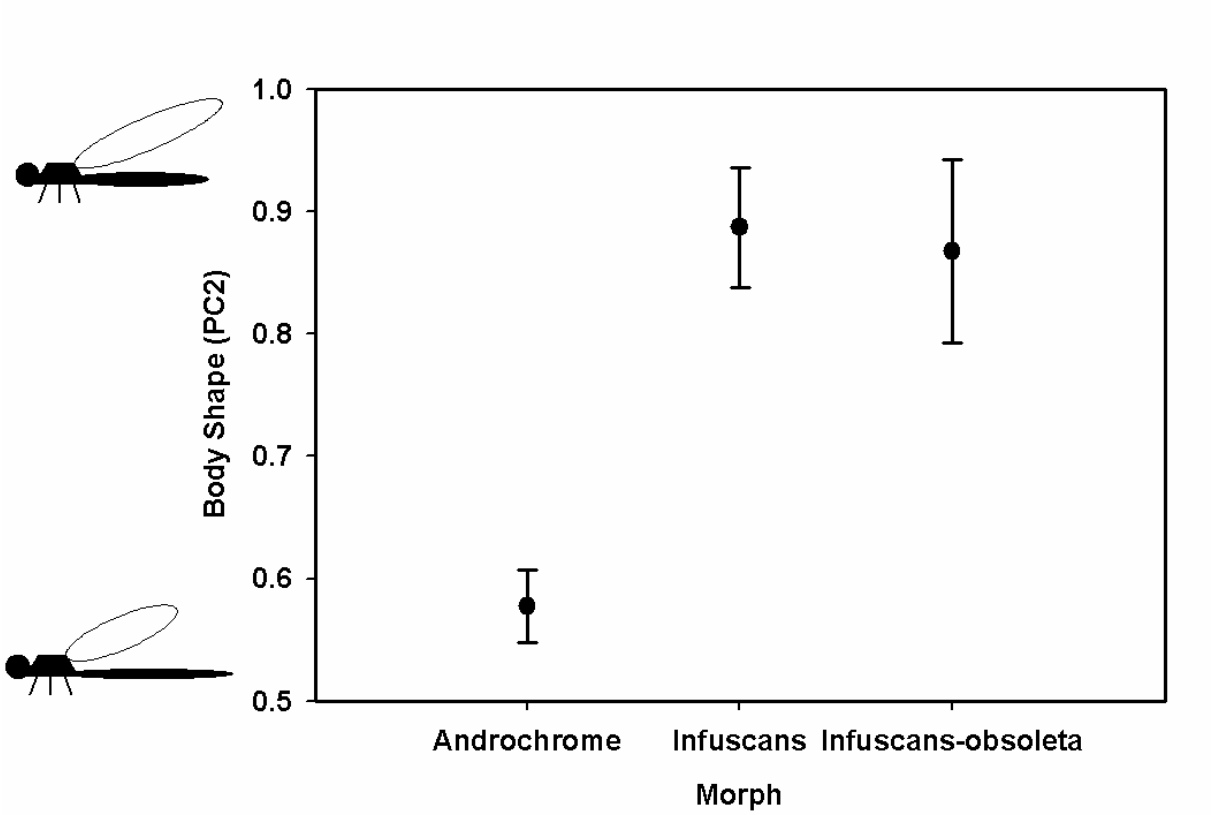


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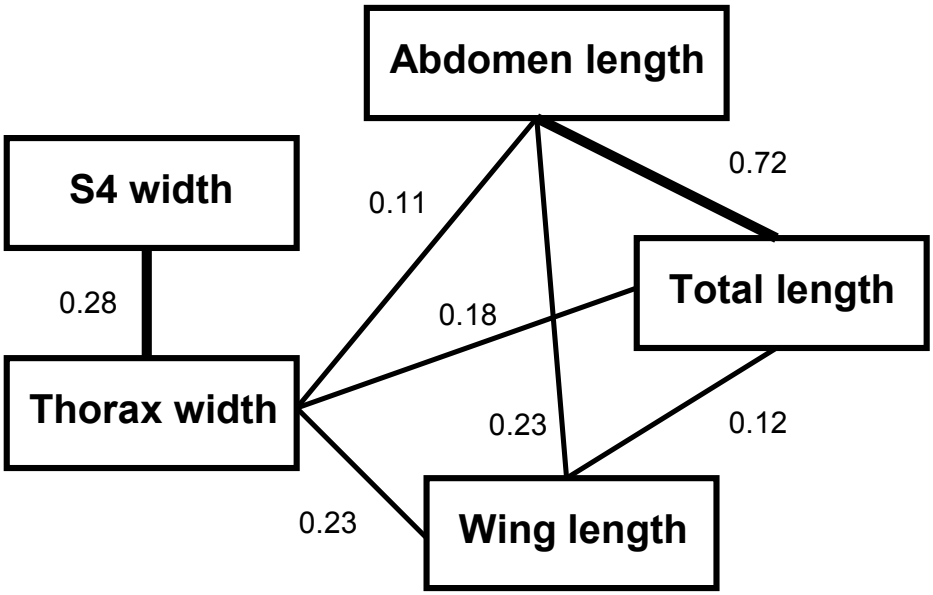
604 Figure 2B




Males

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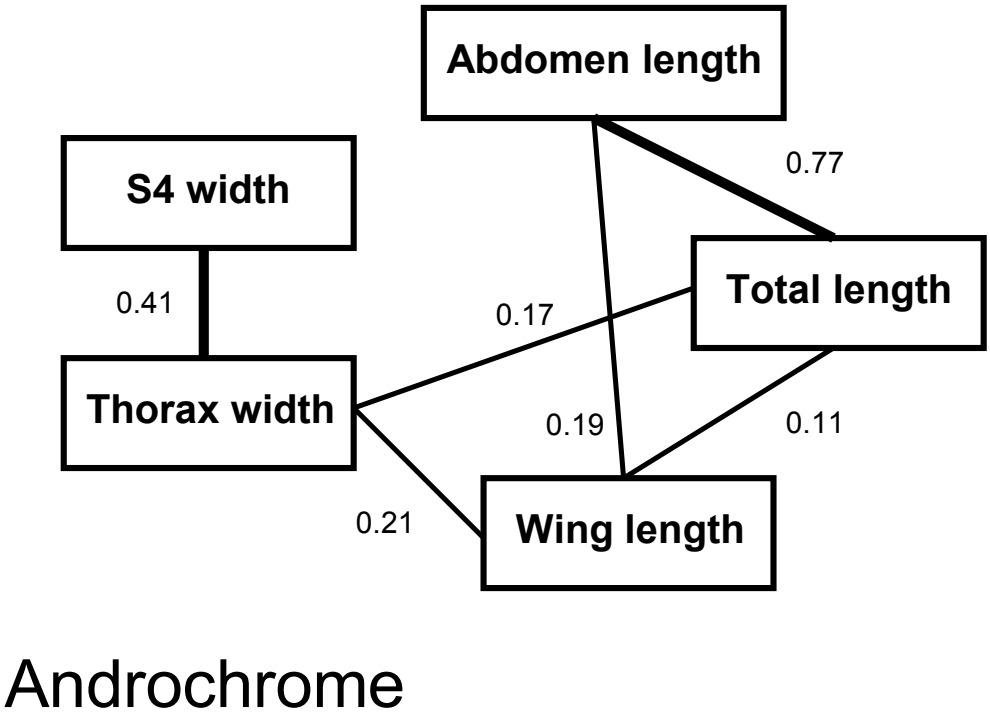
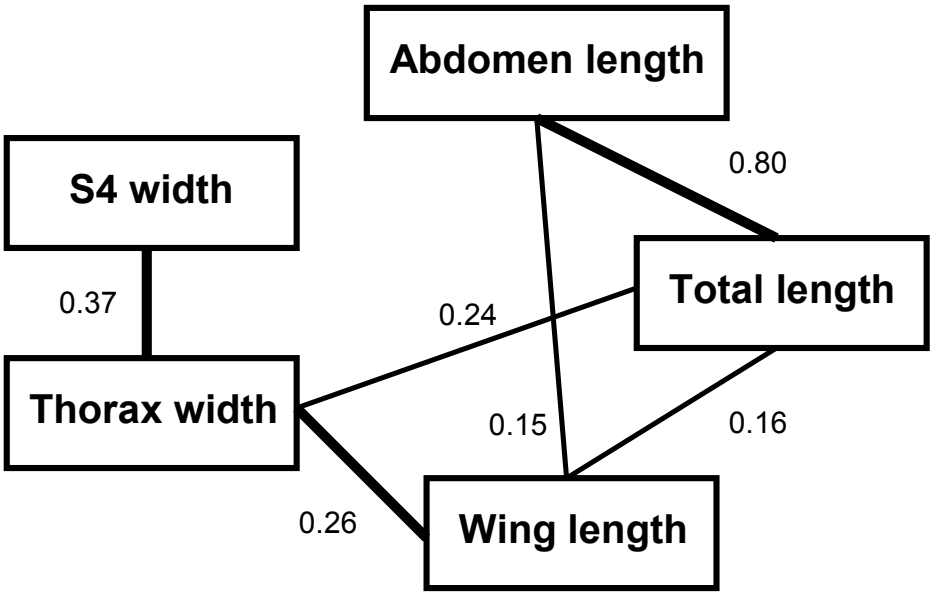
606 Figure 3A




607

608 Figure 3B




Infuscans

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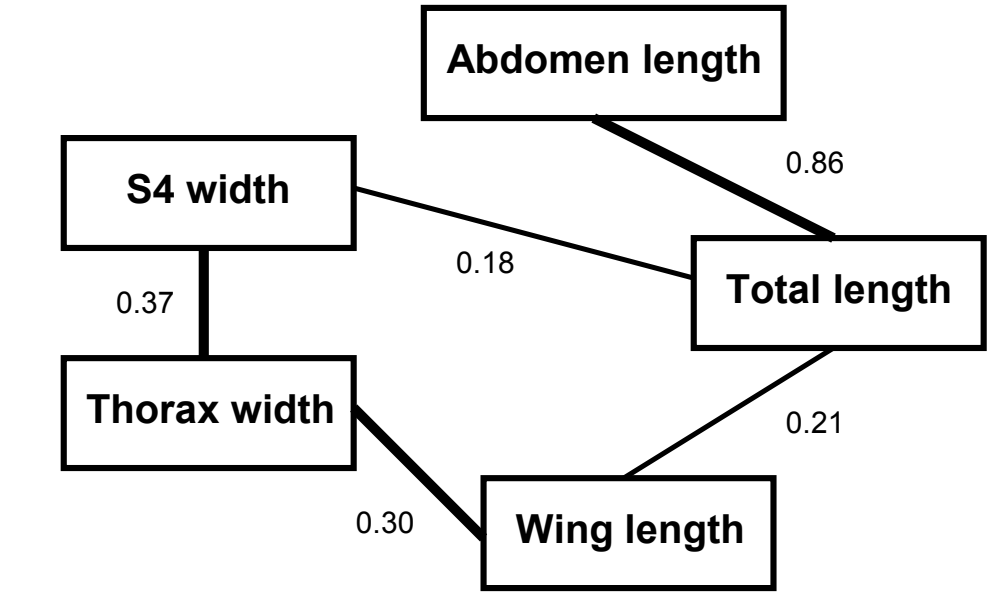
610 Figure 3C

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612 Figure 3D

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