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Ontogeny of sexual dimorphism and phenotypic integration in heritable morphs

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Running title: Sexual dimorphism and phenotypic integration in heritable morphs

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1 **Abstract**

2

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

23

24 Recent years have witnessed an increased interest in the relationship between development
25 and phenotype, and the problem of how integrated phenotypes evolve (West-Eberhard, 2003;
26 Pigliucci & Preston, 2004). This problem is particularly interesting in the context of heritable
27 phenotypic polymorphisms, in which distinct alternative phenotypes maintain their integrity
28 and multitrait differences, despite being controlled by, in many cases, only one or a few
29 genetic loci (Sinervo & Lively, 1996; Shuster & Sassaman, 1997; Sinervo *et al.*, 2000;
30 Svensson *et al.*, 2001; Svensson *et al.*, 2005; Leimar, 2005). There are many conceptual
31 similarities between the persistence of such multiple alternative phenotypes, or morphs, and
32 the evolution of gender differences and sexual dimorphism. Research on sexual size
33 dimorphism has recently focused on its developmental origins. Investigation of how the sexes
34 differ in growth rates and development time has shown that these factors can result in either
35 the enhancement or suppression of adult dimorphism (Badyaev *et al.*, 2001b; Badyaev, 2002).
36
37 In addition, recent theoretical work has suggested that the evolution of sexual dimorphism or
38 heritable polymorphism may be as likely an outcome of disruptive selection as the splitting
39 and evolutionary branching of a population into different species (Bolnick & Doebeli, 2003).
40 In both cases, intraspecific divergence between phenotypes is constrained by the process of
41 genetic recombination and genetic correlations between sexes or morphs (Rice &
42 Chippindale, 2001; Sinervo & Svensson, 2002).
43
44 Although evolutionary developmental biology (“evo-devo”) is a rapidly growing discipline,
45 most research in this area is still focused on classical model organisms such *Drosophila* and
46 *Danio* (Arthur, 2002). Relatively little work has been performed using non-model organisms
47 in ecologically relevant contexts, which has consequently stimulated a recent interest in
48 “ecological and evolutionary developmental biology”, or “eco-evo-devo” (Gilbert, 2001).

49 Here we present the results from a study on the links between larval development and adult
50 phenotype in a non-model organism, a polymorphic damselfly. Genetic colour polymorphism
51 is very common in damselflies but is also present in many other taxa, so our study should
52 have implications beyond our particular study species.

53

54 Our study species, *Ischnura elegans*, has three female colour morphs. Previous work revealed
55 differences between the adult female morphs in fecundity (Svensson *et al.*, 2005; Svensson &
56 Abbott, 2005) and emergence time (Abbott & Svensson, 2005). The female morphs in *I.*
57 *elegans* are maintained by frequency-dependent male-female mating interactions, in which a
58 morph's fecundity decreases as it becomes more common in the population (Svensson &
59 Abbott, 2005). This effect arises because males are thought to form a search image towards
60 common female morphs, which leads to a form of apostatic selection in which common
61 morphs suffer disproportionately from excessive male mating harassment (Fincke, 2004;
62 Svensson *et al.*, 2005). Although researchers have suggested that there may also be
63 differences between the morphs in the larval stage (Cordero, 1992a; Cordero *et al.*, 1998), we
64 are not aware of any studies by other researchers that have investigated this possibility.
65 Differences in emergence and development time between the morphs (Abbott & Svensson,
66 2005) imply that there should be morph-related differences expressed in the larval stage. This
67 motivated us to investigate the differences in larval growth rate and body shape and their links
68 to phenotypic differences in the adult stage, and hence evidence for phenotypic integration
69 between growth rate, shape and color of the morphs (phenotypic integration, as defined by
70 Pigliucci (2003), is "the pattern of functional, developmental and/or genetic correlation
71 (however measured) among different traits in a given organism"). We also present data on the
72 ontogeny of sexual dimorphism in this species. One of our goals with this study is to integrate
73 the study of sexual dimorphism with the study of the developmental origins of heritable

74 morphs, a synthesis that is clearly needed and in which only the first steps have recently been
75 taken (Badyaev, 2002; West-Eberhard, 2003; Sinervo and Svensson, 2004).

76

77 **Materials and methods**

78

79 Study species

80

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

91

92 The development of the female morphs of *I. elegans* is controlled by a single locus with three
93 alleles, similar to the closely related species, *I. graellsii* (Cordero, 1990; Sánchez-Guillén *et*
94 *al.*, 2005). The three alleles form a dominance hierarchy, with the A-allele being dominant to
95 the I- and IO-alleles, the I-allele recessive to the A-allele but dominant to the IO-allele, and
96 the IO-allele recessive to both the other alleles (A>I>IO, Sánchez-Guillén *et al.*, 2005).

97 Although larvae of both sexes and adult males all carry the morph alleles, the colour morphs
98 are only expressed in adult females, hence this is both a stage- and sex-limited polymorphism.

99

100 Morphological measurements

101

102 We collected eggs from damselflies from a natural population, Vombs Vattenverk, outside
103 Lund, in southern Sweden in the summer of 2002. We intended to collect eggs from this
104 population only, but it proved impossible to obtain a balanced data set in this way, due to
105 insufficient numbers of the rarest morph (*Infuscans-obsolata*). Because of this, some clutches
106 of eggs (14 out of a total of 81 clutches) came from females captured at some of the other 13
107 populations we have investigated (see Svensson *et al.*, 2005; Lomma, Hoftorupssjön, Höje å
108 6, Höje å 7, Höje å 14, Flyinge 30A3, and Genarp).

109

110 Mature females of all three morphs were brought back to the laboratory and placed in
111 ovipositoria, small containers with damp filter paper at the bottom. After 48 hours the
112 females were removed and the eggs stored in water until they hatched. After hatching, larvae
113 were transferred to large plastic containers and fed with brine shrimp (*artemia*) daily. We
114 transferred up to ten larvae from each family to individual enclosures within the plastic
115 containers approximately one month after hatching, in order to prevent cannibalism. If more
116 than 10 individuals from the same family were available, the extra individuals were kept but
117 are not included in the analysis of growth trajectories. The individual enclosures contained
118 wooden perches for damselflies to crawl up during emergence.

119

120 Larvae were kept under a constant temperature and light regime (temperature: 17°C, light
121 regime: 12:12) and were maintained in the lab until emergence next spring (2003).

122 Individuals in the lab emerged several months earlier than individuals in the field (in January-
123 May of 2003, rather than May-August), which is probably an effect of temperature rather than

124 photoperiod (de Block & Stoks, 2003). Though temperature affects overall timing of
125 emergence, it does not appear to affect the relative emergence times of the morphs, the sexes,
126 or their final size and shape, since a repetition of the same experiment the following year
127 using two different temperature treatments (12°C and 21°C) did not show any significant
128 effects of temperature on these measurements (Abbott, unpublished data). Once they had
129 been transferred to the individual containers, each larva was given a unique identification
130 number and measured under a light microscope once every 3-4 weeks until emergence. We
131 measured total length (excluding gills), abdomen length, thorax width, width of the 4th
132 segment of the abdomen (S4), and wing pad length (because damselflies are not
133 holometabolous wing development begins in the larval stage), and also determined the sex of
134 the larva by examination of the underside of the abdomen. Damselfly larvae go through
135 several instars before reaching maturity and therefore grow in stages. This means that some
136 individuals might not have reached the next instar between measurement times and should
137 therefore have remained the same size. In a few cases size measurements decreased slightly
138 between measurement times. We then assumed that this was due to measurement error, and
139 took the average of both these measurements.

140

141 Adults were measured and, in the case of females, marked for identification and placed in
142 50*50*50cm insectaria containing water and *Drosophila* until their morph could be
143 determined (no more than 25 females were housed in an insectarium at a time). We measured
144 the same traits in adults as in larvae (total length, abdomen length, thorax width, S4 width,
145 and wing length).

146

147 Statistics

148

149 Principal components analysis was performed on larval measurements, and the first two
150 components were found to be suitable for further analysis. After the larvae had been moved
151 into the individual enclosures we started recording individual mortality.
152
153 Ontogenetic changes in size and shape (PC1 and PC2) were investigated using repeated
154 measures (PROC MIXED, SAS, Littell *et al.*, 1996). The correct covariance structure was
155 determined by comparing the Akaike Information Criterion (AIC). We investigated the
156 effects on PC1 and PC2 of the fixed factors Maternal morph and Sex in all individuals, and of
157 Own morph in females only. We also investigated whether there was any difference in
158 developmental trajectories between individuals that managed to emerge successfully and
159 those that did not. Family was included as a random factor in all analyses, except of the effect
160 of own morph on PC1, to control for non-independence of siblings (Fry, 1992). It was
161 impossible to include Family as random factor in the analysis of the effect of Own morph on
162 PC1, probably because this subset of the data was too unbalanced, so in this case, Family was
163 included as a fixed factor instead. Two-way interactions between all factors (except
164 Sex*Morph since males are monomorphic and Morph*Emergence since we could not
165 determine morph for females that died in the larval stage) were also tested but this did not
166 change the results, so for simplicity interaction effects will not be presented here. An analysis
167 of the effect of Maternal morph on PC2 for males only was also carried out, to see whether
168 differences between offspring of the morphs were due to biased sex ratios.
169
170 We also looked at the effects of Sex, Maternal morph, Own morph, and whether the
171 individual emerged successfully on morphology in the last instar using a mixed model with
172 Family as a random factor.

173

174 We analysed the probability of emerging according to Sex and Maternal morph with Family
175 as a random factor using a generalized linear model (GLIMMIX macro in SAS, Littell *et al.*,
176 1996) with binomial error and logit link function. This was done to investigate if differences
177 between individuals that emerged and those that did were possibly confounded by differences
178 in survival rates between the sexes, between offspring of the female morphs, or between
179 families,

180

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

190

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

195

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

198

199 **Results**

200

201 Mortality

202

203 Mortality was relatively modest; 28% of all individually tracked larvae died (227/806
204 individuals). By plotting a histogram of wing pad lengths we were able to identify when
205 individuals had reached the last instar (in this case, when wing pad length was greater than
206 3.5mm (Benke, 1970)). Most of these individuals (174/806, or 21%) died when in early
207 instars, not long after being moved into the individual enclosures, probably as a result of the
208 changed environmental conditions. These individuals were excluded from all further
209 analyses. The remainder (53/806, or 7%) died in the last instar, close to or during emergence.
210 We believe that it is unlikely that individuals that had survived several months after being
211 moved into the individual enclosures suddenly died because of the conditions in the lab, and
212 so we assumed that this later mortality was related to problems during emergence. Probability
213 of emerging was not related to Maternal morph ($F_{2, 618}=0.12$, $P=0.8884$), Sex ($F_{1, 628}=0.68$,
214 $P=0.4110$), or Family ($F_{78, 628}<0.01$, $P>0.99$) so differences between individuals that emerged
215 and those that did not are not a result of differential mortality between these groups.

216

217 Larval morphology

218

219 The principal components analysis of larval morphology indicated that PC1 was a measure of
220 overall size, which accounted for most of the variation in morphology (96%). There was also
221 a minor component of the variation (2.7%) which was related to variation in shape, such that
222 positive values of PC2 indicate a longer abdomen and shorter wings, while negative values
223 indicate a shorter abdomen and longer wings (Table 1). The last three PCs accounted for less

224 than 1% of the variation each. Although PC2 accounted for a small part of the total variation,
225 this is probably due to the nature of the data set (a growth series). According to Jackson
226 (1991), in cases where the first principal component accounts for an overwhelming part of the
227 variation in the data it may still be appropriate to include other PCs in the analysis as long as
228 they are informative, i.e. the PC has an eigenvalue unequal to all subsequent PCs. Since the
229 difference in eigenvalue between PC2 and PC3 is almost three and a half times greater than
230 the difference in eigenvalue between PC3 and PC4 (0.093 versus 0.027), we believe that PC2
231 is actually capturing an important and informative, if relatively small, part of the total
232 variation. In addition, PC2 in the larval and adult stages both indicate a negative relationship
233 between wing length and abdomen length, as does PC2 in an analysis of morphology of field-
234 caught adults (Abbott and Svensson, unpublished data), all of which suggests that the pattern
235 seen in PC2 in the larval stage is informative.

236

237 We found significant effects of all factors tested on body size (PC1) and body shape (PC2).
238 In these analyses, significant effects indicate differences between the equations of the best-fit
239 lines which describe the data. Main effects correspond to differences in intercept, the
240 factor*time interactions to differences in slope, and the factor*time² interactions to differences
241 in curvature (Littell *et al.*, 1996). For body size, we found that females had a higher growth
242 rate than males, that offspring of Infuscans-obsolata females had a higher growth rate than the
243 offspring of the other two morphs, and that Androchrome females had a slightly higher
244 growth rate than females of the other two morphs (Table 2, Figure 1A-C). Individuals that
245 managed to emerge had a higher growth rate than individuals that did not emerge (Table 2,
246 Figure 1D). Females in the last instar were significantly larger than males in the last instar
247 ($F_{1, 632}=141.70$, $P<0.0001$), Androchrome females were significantly larger than Infuscans
248 females in the last instar ($F_{2, 229}=5.91$, $P=0.0032$), and individuals that emerged successfully

249 were larger in the last instar than individuals that did not emerge ($F_{1, 628}=13.11$, $P=0.0003$;
250 Table 4).

251

252 For body shape, we found that males start off with shorter abdomens and longer wing pads
253 than females, but that they end up with longer abdomens and shorter wing pads (Table 3,
254 Figure 2A). We also found that offspring of Infuscans-obsolata females have longer
255 abdomens and shorter wing pads than the offspring of the other two morphs (Figure 2B). This
256 pattern held even when only males were included in the analysis (quadratic time effect of
257 Maternal morph: $F_{2, 258}=318.27$, $P<0.0001$; pattern is the same as in Figure 2B), so this reflects
258 a real effect of Maternal morph on offspring morphology which cannot simply be a result of
259 biased sex or morph ratios in offspring. Individuals that managed to emerge initially had
260 shorter abdomens and longer wing pads (lower values of PC2) than individuals that did not,
261 with the reverse pattern later in development (Figure 2D). This was also evident in the last
262 instar, where individuals that emerged successfully had longer abdomens and shorter wing
263 pads than individuals that did not emerge ($F_{1, 628}=19.43$, $P<0.0001$; Table 4). This suggests the
264 existence of internal selection on body shape. There was also an effect of Own morph on body
265 shape, with rank order of the different morphs changing several times over development
266 (Figure 2C). The difference between the morphs in the final instar approached significance,
267 with Androchrome females having a more male-like morphology (higher value of PC2) than
268 the other two morphs ($F_{2, 229}=2.92$, $P=0.0560$; Table 4).

269

270 Laboratory-raised adults

271

272 Similar to the analysis of larval morphology, PCA on adult morphology resulted in PC1 as a
273 measure of overall size which accounted for 60.1% of the variation. PC2 was found to be a

274 measure of shape which accounted for 26.5% of the variation, where positive values indicate
275 relatively longer abdomens, but shorter wings and narrower S4, and negative values indicate
276 relatively shorter abdomens, with longer wings and wider S4 (Table 5). All other PCs
277 accounted for a relatively small part of the variation (data not shown).

278

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

286

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

292

293 **Discussion**

294

295 Sexual dimorphism and heritable polymorphism in *I. elegans* are characterized by phenotypic
296 integration of colour and morphology (this study), and differences in development time
297 between different phenotypes (Abbott & Svensson, 2005). In addition, development rate
298 interacts with development time to influence size. In the sexes, size differences are enhanced

299 by this interaction, while in the morphs, size differences are instead suppressed by the same
300 type of interaction.

301

302 Size differences

303

304 Sexual size dimorphism in vertebrates can result from differences in development time,
305 development rate or both these factors acting jointly (Badyaev, 2002). Here we have shown
306 that females have a higher larval growth rate than males (Figure 1A), and were larger in the
307 final instar (Table 4) and as adults (Figure 3). In a previous analysis of data from the same
308 laboratory-raised population (Abbott & Svensson, 2005), we have shown that males emerged
309 earlier than females (protandry). Thus, sexual differences in development time and
310 development rate are acting jointly, and in the same direction, to promote sexual size
311 dimorphism in *I. elegans*.

312

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

324 results indicate that pleiotropic, physiological effects of the morph locus may also be
325 involved.

326

327 Shape differences

328

329 Shape differences between the sexes and the morphs were generally consistent between the
330 different life stages. In the adult stage, males have relatively longer abdomens, shorter wings,
331 and narrower S4 (Figure 4A) than females. This is consistent with their shape in the final
332 instar, where males have longer abdomens and relatively shorter wing pads than females
333 (Table 4). Similarly, the Infuscans-obsolata morph was the most divergent morph in both
334 stages, although in the adult stage this was evident as an effect of the female's Own morph
335 (Figure 4B), while in the larval stage it was due to the effect of Maternal morph (Figure 2B,
336 comparing parental and offspring traits is a standard quantitative-genetic approach; see Abbott
337 & Svensson, 2005 for details).

338

339 Both size and shape differences seem to have additive genetic components, as indicated by the
340 significant effects of the factor Family on both PC1 (Tables 2 and 6) and PC2 (Tables 3 and
341 7). Body length has previously been demonstrated to be heritable in a related species
342 (Cordero, 1992b). Our findings that most of this genetic variation is aligned along the size
343 axis with less variation in shape is consistent with many other quantitative-genetic studies on
344 other organisms (Schluter, 1996). The phenotypic correlations found here also confirm that
345 larval and adult size and shape are related (Table 8), which has previously been shown for
346 size (Harvey & Corbet, 1985; Banks & Thompson, 1987; Cordero, 1992b). In the closely
347 related damselfly genus *Enallagma*, larval phenotypic traits influenced by selection imposed

348 by different aquatic predators such as fish or dragonfly larvae may show a correlated response
349 to selection on reproductive traits in the adult stage (Stoks *et al.*, 2003; Stoks *et al.*, 2005).

350

351 Sexual dimorphism

352

353 Adult males of *I. elegans* are both smaller than females and different in shape, as well as
354 being monomorphic for colour (in contrast to the colour polymorphic females). The size
355 difference between the sexes is probably a result of selection for protandry (earlier emergence
356 of males), since males engage in scramble competition for females. Previous field studies
357 have shown that small males may have higher mating success in some populations (Cordero
358 *et al.*, 1997; Carchini *et al.*, 2000). For females, fecundity is likely to be more influenced by
359 body size than by timing of emergence (Cordero, 1991; Morbey & Ydenberg, 2001). Thus,
360 sexual size dimorphism in this species may result from sexually antagonistic selection on
361 body size, with different size optima for males and females (Rice & Chippindale, 2001). The
362 shape differences between the sexes should reflect adaptive differences arising from gender-
363 specific reproductive roles. Males must have relatively long abdomens for completion of the
364 wheel position during mating (Corbet, 1999) and females may have wider abdomens than
365 males in order to accommodate the ovaries. The presence of the ovaries implies that females
366 should be heavier than males of the same length, which may in turn select for longer wings.

367

368 We also note that the maternal morphs also influence the shape of their monomorphic sons
369 (Table 3 and Figure 2B). An analysis of the effect of Maternal morph on PC2 in only males
370 results in the same pattern as seen in Figure 2B, with male offspring of *Infuscans-obsolata*
371 females having the most male-like shape in the larval stage. This may have some implications
372 for ontogenetic sexual conflict between loci affecting overall shape and the morph locus. We

373 have previously argued in a similar vein that there is a conflict between loci for early
374 emergence favouring male protandry and the morph-locus which also influences development
375 time in both males and females (Abbott & Svensson, 2005).

376

377 Phenotypic integration

378

379 The fact that the female morphs in *I. elegans* differ in colour (Askew 1988), shape and
380 development rate (this study), as well as development time (Abbott & Svensson, 2005) and
381 fecundity (Svensson *et al.*, 2005; Svensson & Abbott, 2005), suggests that suites of
382 phenotypic traits are integrated in these morphs. This has some similarities to the adaptive
383 phenotypic integration documented for male secondary sexual characters in several avian
384 taxa, which are thought to be promoted by correlational selection for optimal character
385 combinations (Badyaev *et al.*, 2001a; Badyaev, 2004a; Badyaev, 2004b; McGlothlin *et al.*,
386 2005). Multi-trait differences between the morphs could have been caused by maternal
387 effects, pleiotropy, or linkage disequilibrium (Lynch & Walsh, 1998) due to physical linkage
388 between loci for colour and morphology or which is built up in each generation by
389 correlational selection (Brodie, III, 1992). The data in this study do not allow us to
390 distinguish between these different explanations for the persistence of multi-trait differences
391 between these morphs.

392

393 The general pattern and direction of morph-specific differences in *I. elegans* are consistent
394 with the hypothesis that Androchrome females are male mimics, because they have both
395 male-like melanin patterning, male-like blue coloration, male-like behaviour (Van Gossum *et al.*
396 *al.*, 2001) and they are also more male-like in shape (i. e. high value of PC2, cf. Figure 4). It
397 is possible that these striking and multiple phenotypic similarities between Androchromes and

398 males are simply non-adaptive pleiotropic effects of the allele producing male-like coloration.
399 However, the observed pattern is certainly also consistent with selection to improve male
400 mimicry in Androchromes either through direct selection on shape, or indirectly via selection
401 for more male-like behaviour, such as flight or movement patterns, or as a response to
402 avoiding male mating harassment. For instance, morph-differences in relative wing to
403 abdomen length (i. e. PC2, see Fig. 4B) may affect flight speed or manoeuvrability, and
404 thereby success in escaping unwanted male mating harassment and mating attempts.

405

406 Male mating harassment in Ischnurans is likely to be substantial since females mate with
407 multiple males (Cooper *et al.*, 1996) but only require one insemination to produce as many
408 fertile eggs as females that have mated several times (Sirot & Brockmann, 2001), and more
409 mating attempts are initiated than are carried out (T. Gosden & E. I. Svensson, unpublished
410 data). This harassment may select for different phenotypic female optima, so that females can
411 avoid such harassment by either becoming a more or less perfect male mimic (i. e.
412 Androchromes) or by developing a divergent phenotype in colour and shape (i. e. Infuscans-
413 obsoleta) or by becoming so different that it falls outside the usual range of female
414 phenotypes encountered by males. Interestingly, Infuscans-obsoleta is also the morph that is
415 found least frequently *in copula* in the field, relative to their frequency in the population
416 (Svensson *et al.*, 2005).

417

418 These adaptive explanations for the phenotypic integration in female morphs are consistent
419 with both models and data that indicate intraspecific genetic diversification is an expected
420 outcome of male mating harassment (Gavrilets & Waxman, 2002), particularly if males have
421 visual or other perceptive constraints that force them to develop a search image for only one
422 female morph at a time (Fincke, 2004; Svensson *et al.*, 2005). Such intraspecific divergence

423 has two possible outcomes: it could subsequently promote speciation, or constrain it by
424 eliminating selection pressures for additional divergence through the formation of stable
425 female genetic clusters (polymorphism; Svensson *et al.*, 2005).

426

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

433

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

435

436 We found evidence for fitness consequences on morphology in the larval stage, since
437 individuals that managed to emerge successfully differed in both size and shape (PC1 and
438 PC2) from those that did not. Surprisingly, individuals that emerged started off smaller in size
439 than those that did not (Figure 1D). There are two possible explanations for this pattern,
440 antagonistic pleiotropy and competition. In antagonistic pleiotropy, alleles with positive
441 effects early in development have negative effects later in development (Rose, 1982).

442 Alternatively, there could be differences in competitive ability which are the result of a trade-
443 off between growth rate while under intraspecific competition and growth rate when solitary,
444 since larvae were not moved to individual enclosures until a few weeks after hatching. Such
445 trade-offs between growth rate under crowded and non-crowded conditions have indeed been
446 documented previously in laboratory selection experiments of *Drosophila* (Mueller & Ayala,
447 1981; Mueller, 1988; Borash *et al.*, 1998).

448

449 Since individuals that emerged successfully differed in body shape from those that did not,
450 this suggests that there is selection on body shape in the larval stage. This type of selection
451 could contribute to the build-up of linkage disequilibrium in the female morphs (see above).
452 Individuals that emerged had shorter abdomens and longer wings than those that did not, so
453 there appears to be some sort of internal (“non-ecological”) selection on shape. Internal
454 selection refers to selection that acts on organismal traits independently of ecology (Schwenk
455 & Wagner, 2001). Internal selection caused by developmental problems is more likely in this
456 laboratory study in which predators and other ecological agents of selection can be excluded
457 as mortality causes. The fact that this type of internal selection appeared to favour shorter
458 wings (see Results) is particularly interesting and may indicate that there may be development
459 fitness costs of long wings that may counteract selection for longer wings or larger size at the
460 adult stage (Kingsolver & Pfennig, 2004). The relevance of such selection in the field is
461 unknown, but could be important if mortality due to other causes (such as predation) is
462 random with respect to an individual’s ability to emerge successfully.

463

464 Conclusions

465

466 We have found evidence of phenotypic integration of many traits in the female morphs, such
467 as colour pattern, morphology, developmental rate (this study), development time (Abbott &
468 Svensson, 2005), and fecundity (Svensson *et al.*, 2005; Svensson & Abbott, 2005). These and
469 other results reveals the similarities between the development of morphological differences of
470 heritable morphs in *Ischnura elegans* and the development of sexual dimorphism in both this
471 insect and vertebrate species (Badyaev, 2002). Both these phenomena can be analysed and
472 understood in terms of the interactive effects of developmental rate and development time,

473 two factors which can enhance or counteract each other during the course of development. We
474 are currently investigating sexual dimorphism and phenotypic integration in field-caught
475 adults, genetic correlations and heritability of morphological traits, and are also analyzing
476 larval morphology using geometric morphometric techniques. Other interesting questions for
477 further research include the relative importance of maternal effects, pleiotropy, and linkage
478 disequilibrium (of linked or unlinked loci) in producing morph-related differences, and the
479 effect of competition on development of adult phenotypes.

480

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Table 1: Factor loadings for PC1 and PC2 in the larval stage. PC1 is a measure of overall size, while PC2 mostly represents a trade-off in wing length and abdomen length.

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

Table 2: Table of repeated measures analysis of effects of Sex, Maternal morph, Own morph, and Emergence on PC1 (body size) in the larval stage. Family was included as a random factor in all analyses, except Own morph, where it is a fixed factor (see text). A significant effect of the factor indicates significant differences in the intercepts of the trajectories, a significant interaction between the factor and time indicates significant differences in the slope of the trajectories, and a significant interaction between the factor and time² indicates significant differences in the curvature of the trajectories. For fixed effects (Maternal morph, Sex, Own morph, Emergence) the test statistic is F, for random effects (Family) it is Z.

Effect	Num Df	Den Df	F	Z	P-value
Sex (N=632):					
Sex	2	185	6.46		0.0019
Sex*time	2	565	5567.42		<0.0001
Sex*time ²	2	575	169.73		<0.0001
Family	78			3.60	0.0002
Maternal morph (N=622):					
Maternal morph	3	105	2.81		0.0429
Maternal morph*time	3	562	3749.10		<0.0001
Maternal morph*time ²	3	574	124.71		<0.0001
Family	77			3.54	0.0002
Own morph (females only, N=229):					

Own morph	3	139	0.99	0.3973
Own morph*time	3	203	1395.54	<0.0001
Own morph*time ²	3	199	47.45	<0.0001
Family	76	53.3	1.91	0.0066
Emergence (N=628):				
Emergence	2	232	3.17	0.0440
Emergence*time	2	543	5586.27	<0.0001
Emergence*time ²	2	553	172.05	<0.0001
Family	78		3.51	0.0002

Table 3: Table of repeated measures analysis of effects of Sex, Maternal morph, Own morph, and Emergence on PC2 (body shape) in the larval stage. Family was included as a random factor in all analyses. A significant effect of the factor indicates significant differences in the intercepts of the trajectories, a significant interaction between the factor and time indicates significant differences in the slope of the trajectories, and a significant interaction between the factor and time² indicates significant differences in the curvature of the trajectories. For fixed effects (Maternal morph, Sex, Own morph, Emergence) the test statistic is F, for random effects (Family) it is Z.

Effect	Num Df	Den Df	F	Z	P-value
Sex (N=632):					
Sex	2	151	2.45		0.0895
Sex*time	2	546	13.82		<0.0001
Sex*time ²	2	561	1148.64		<0.0001
Family	78			4.40	<0.0001
Maternal morph (N=622):					
Maternal morph	3	86	0.48		0.6943
Maternal morph*time	3	537	9.10		<0.0001
Maternal morph*time ²	3	551	728.88		<0.0001
Family	77			4.34	<0.0001
Own morph (females only, N=229):					

Own morph	3	185	2.33	0.0761
Own morph*time	3	215	4.81	0.0029
Own morph*time ²	3	218	417.54	<0.0001
Family	76		3.37	0.0004
Emergence (N=628):				
Emergence	2	229	0.22	0.7988
Emergence*time	2	531	20.04	<0.0001
Emergence*time ²	2	541	1217.44	<0.0001
Family	78		4.39	<0.0001

Table 4: LS means of A) morphological measurements and PCs in the final instar according to Sex, Maternal morph, Own morph, and Emergence and B) morphological measurements in the adult stage according to Sex and Own morph. All values were calculated from mixed models with family as a random factor and are presented in the form Mean (SE).

Morphological measurements (total length, abdomen length, thorax width, width of the 4th segment of the abdomen, and wing pad length) are in mm.

A)

Sex:

	Female	Male
Length	15.13 (0.07)	14.70 (0.07)
Abdomen	10.12 (0.04)	10.92 (0.04)
Thorax	2.37 (0.007)	2.28 (0.006)
S4	1.45 (0.005)	1.36 (0.005)
Wing	4.47 (0.017)	4.20 (0.016)
PC1	1.50 (0.016)	1.31 (0.016)
PC2	-0.87 (0.032)	-0.84 (0.030)

Maternal morph:

	Androchrome	Infuscans	Infuscans-obsoleta
Length	14.90 (0.10)	14.83 (0.12)	14.96 (0.13)
Abdomen	10.00 (0.05)	9.99 (0.07)	10.06 (0.07)
Thorax	2.33 (0.009)	2.31 (0.011)	2.32 (0.012)
S4	1.41 (0.006)	1.40 (0.008)	1.40 (0.009)

Wing	4.34 (0.022)	4.29 (0.029)	4.35 (0.029)
PC1	1.40 (0.022)	1.37 (0.029)	1.42 (0.030)
PC2	-0.87 (0.032)	-0.83 ((0.052)	-0.85 (0.053)

Own morph:

	Androchrome	Infuscans	Infuscans-obsoleta
Length	15.40 (0.09)	14.95 (0.11)	14.98 (0.23)
Abdomen	10.27 (0.05)	10.04 (0.06)	10.02 (0.12)
Thorax	2.39 (0.009)	2.35 (0.010)	2.37 (0.022)
S4	1.46 (0.006)	1.44 (0.006)	1.46 (0.014)
Wing	4.54 (0.022)	4.45 (0.024)	4.44 (0.052)
PC1	1.57 (0.022)	1.47 (0.025)	1.49 (0.053)
PC2	-0.82 (0.036)	-0.93 (0.040)	-0.85 (0.086)

Emergence:

	Unsuccessful	Successful
Length	14.37 (0.14)	14.94 (0.06)
Abdomen	9.70 (0.08)	10.04 (0.04)
Thorax	2.27 (0.014)	2.32 (0.006)
S4	1.39 (0.011)	1.40 (0.004)
Wing	4.29 (0.039)	4.33 (0.015)
PC1	1.29 (0.034)	1.41 (0.015)
PC2	-1.10 (0.061)	-0.84 (0.027)

B)

Sex:

	Female	Male
Length	30.07 (0.10)	30.06 (0.09)
Abdomen	23.54 (0.08)	23.78 (0.08)
Thorax	2.19 (0.007)	2.09 (0.006)
S4	0.73 (0.004)	0.62 (0.003)
Wing	18.77 (0.06)	17.10 (0.05)

Own morph:

	Androchrome	Infuscans	Infuscans-obsoleta
Length	30.20 (0.13)	30.06 (0.15)	29.61 (0.30)
Abdomen	23.62 (0.11)	23.58 (0.12)	23.10 (0.25)
Thorax	2.20 (0.010)	2.18 (0.011)	2.21 (0.022)
S4	0.72 (0.005)	0.73 (0.005)	0.75 (0.011)
Wing	18.85 (0.08)	18.75 (0.09)	18.59 (0.19)

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

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

Table 6: Table of mixed model analysis of effects of Sex, Maternal morph and Own morph on PC1 (body size) in the adult stage. Family was included as a random factor in all analyses. Maternal morph and Sex were included in the first analysis (all offspring), and Maternal morph and Own morph in the second (females only). For fixed effects (Maternal morph, Sex, Own morph) the test statistic is F, for random effects (Family) it is Z.

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

Table 7: Table of mixed model analysis of effects of Sex, Maternal morph and Own morph on PC2 (body shape) in the adult stage. Family was included as a random factor in all analyses. Maternal morph and Sex were included in the first analysis (all offspring), and Maternal morph and Own morph in the second (females only). For fixed effects (Maternal morph, Sex, Own morph) the test statistic is F, for random effects (Family) it is Z.

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

Table 8: Table of phenotypic correlations between larval and adult traits. Only correlations between the same trait measured in both stages in the same individual are included (i.e. larval body length in the last instar correlated with adult body length, larval abdomen length in the last instar with adult abdomen length, etc.)

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

Figure legends

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

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

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

Figure 4: Differences in body shape (PC2) in the adult stage between A. Males and females.

Males have relatively longer abdomens, shorter wings, and narrower S4 than females.

B. Females of different morphs. Infuscans-obsolata females were significantly different ($P < 0.05$) from Androchrome and Infuscans females, with relatively shorter abdomens, longer wings and wider S4.

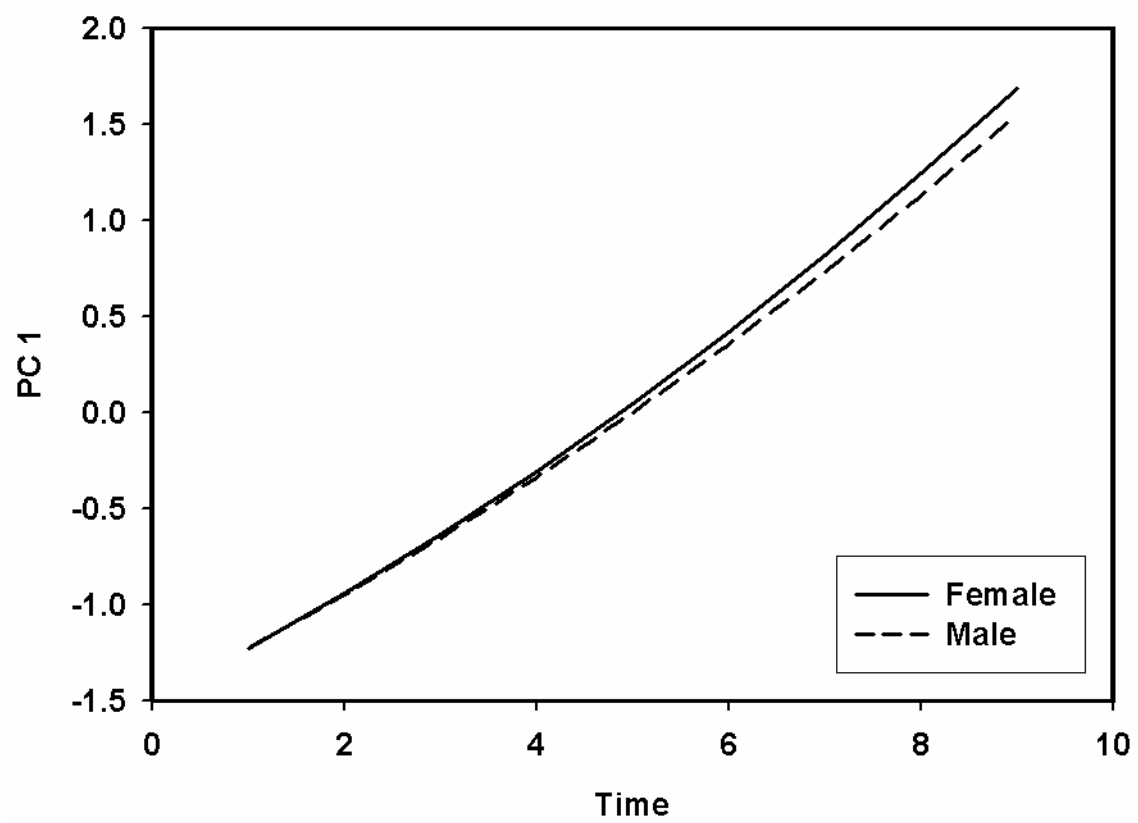


Figure 1A

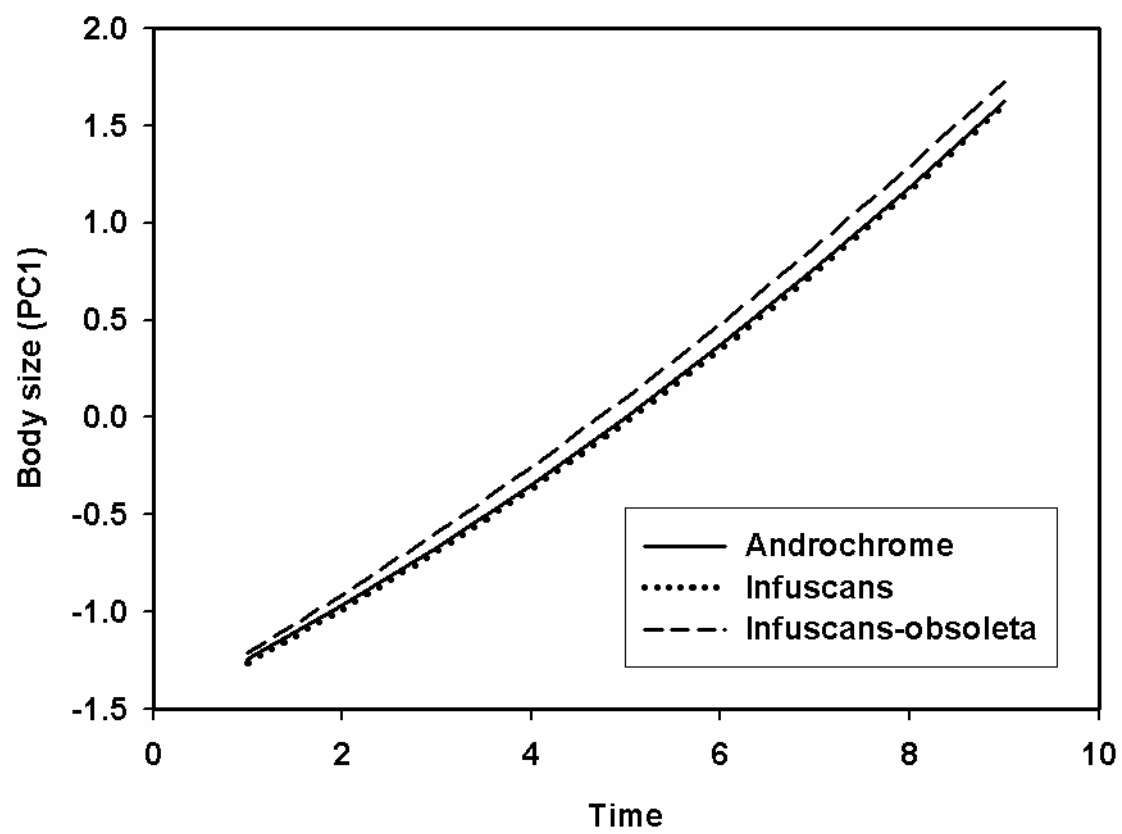


Figure 1B

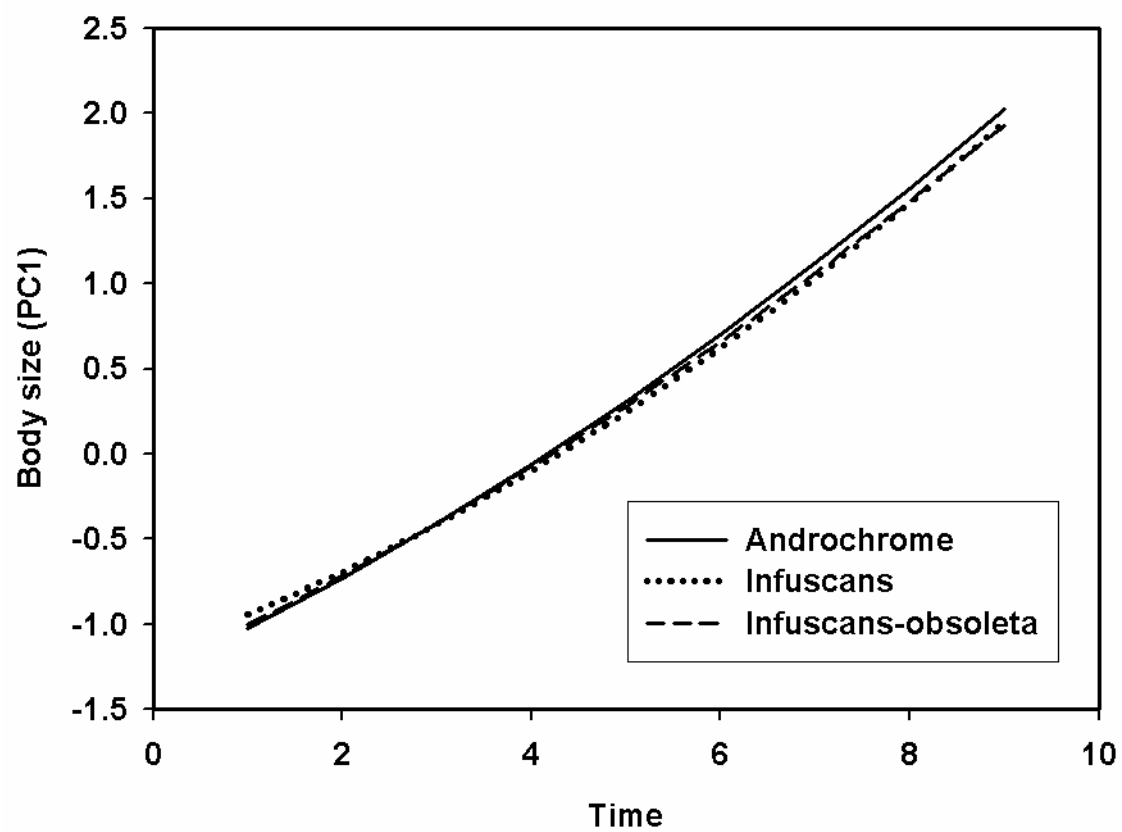


Figure 1C

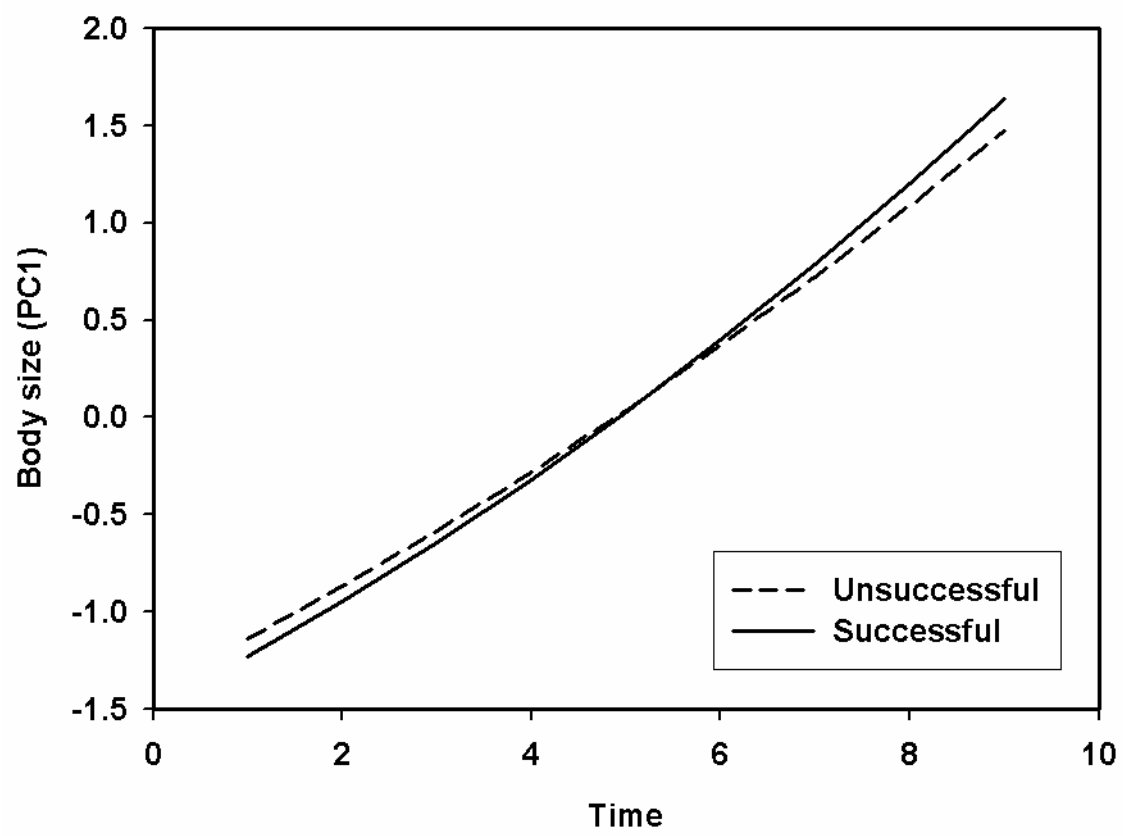


Figure 1D

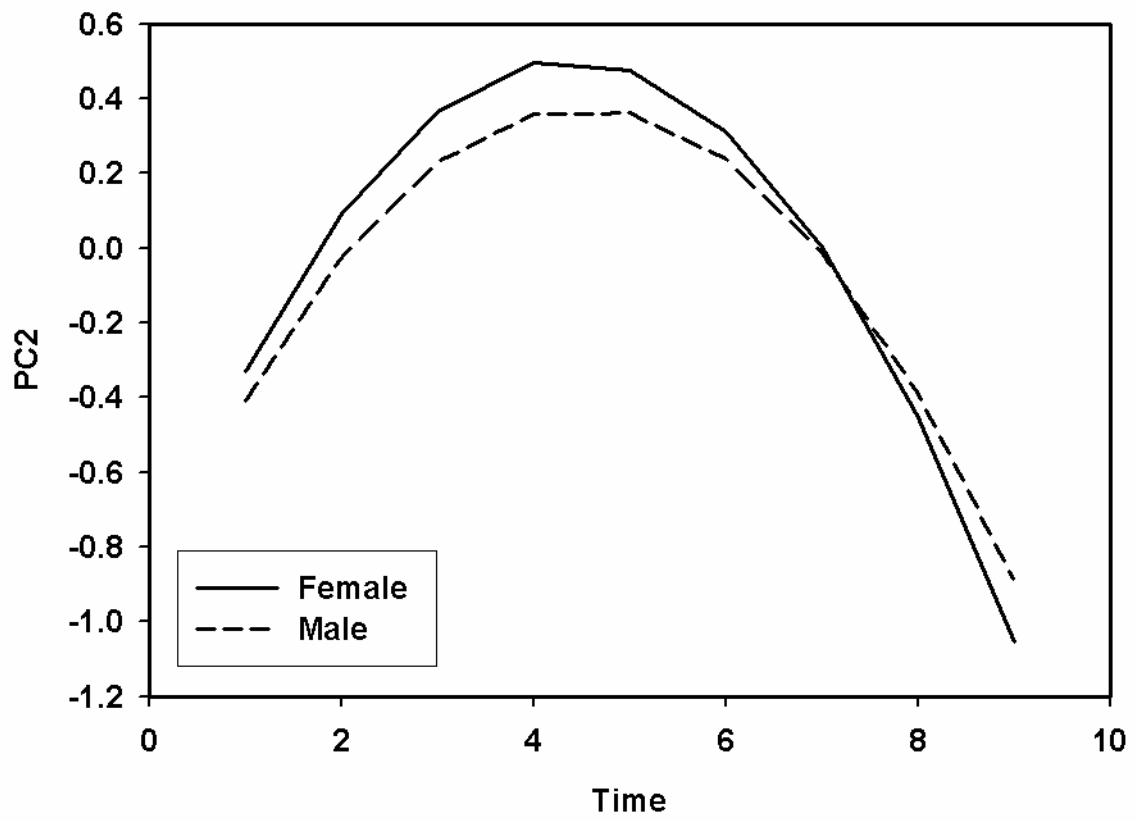


Figure 2A

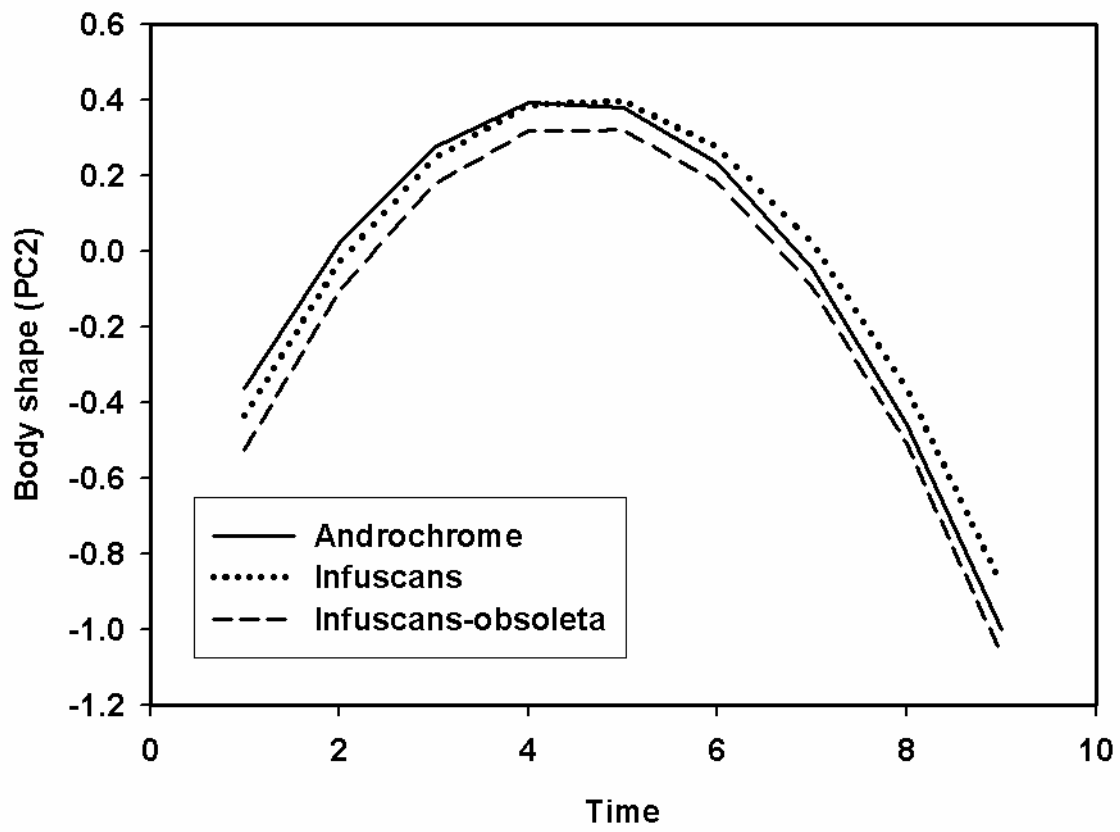


Figure 2B

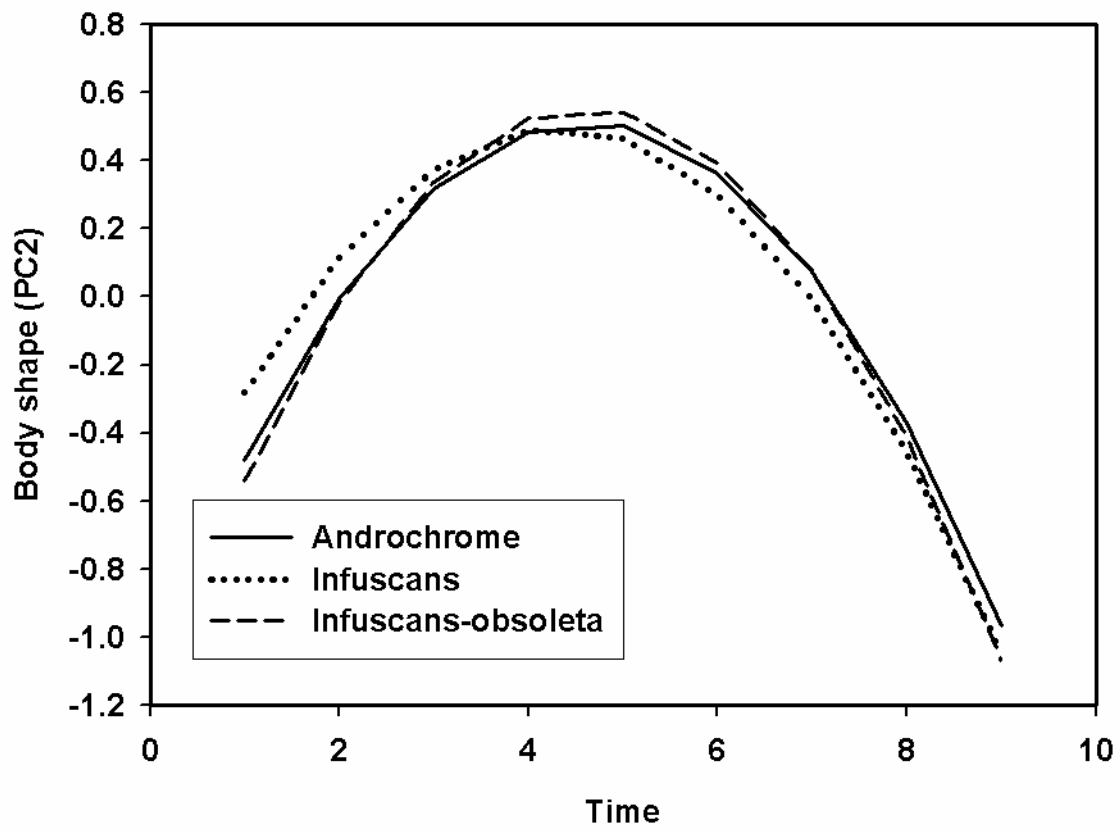


Figure 2C

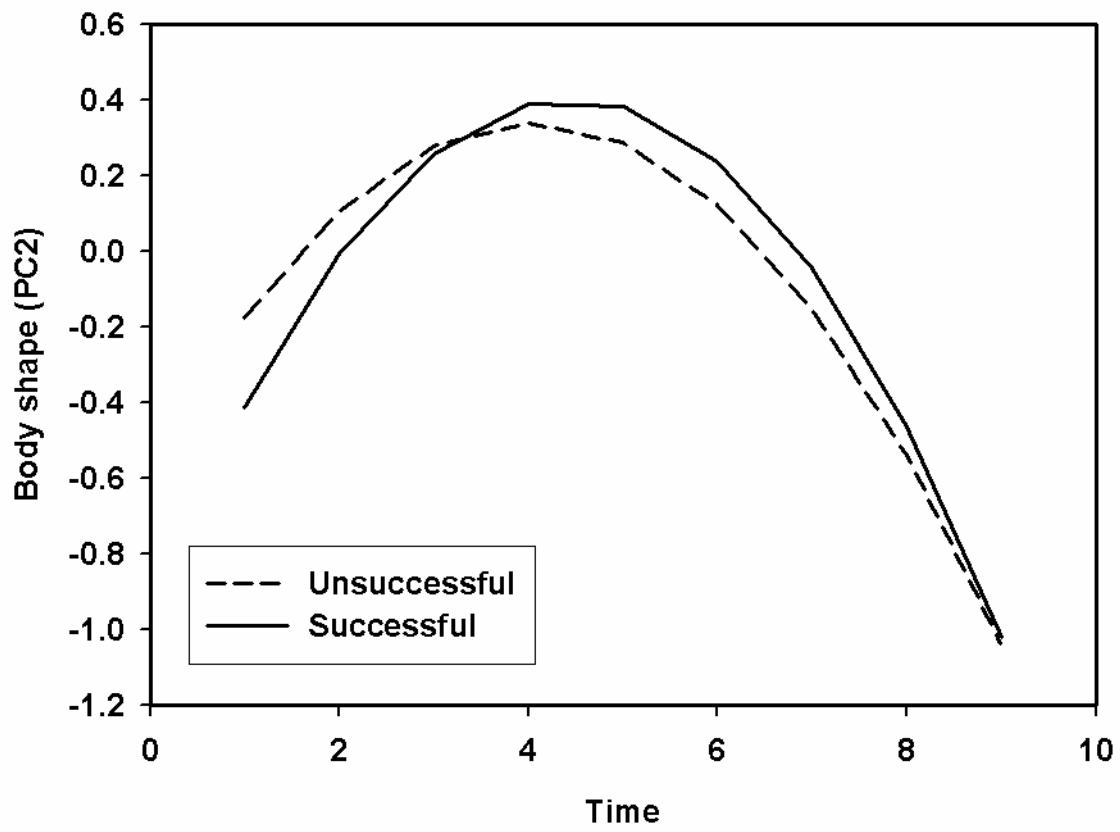


Figure 2D

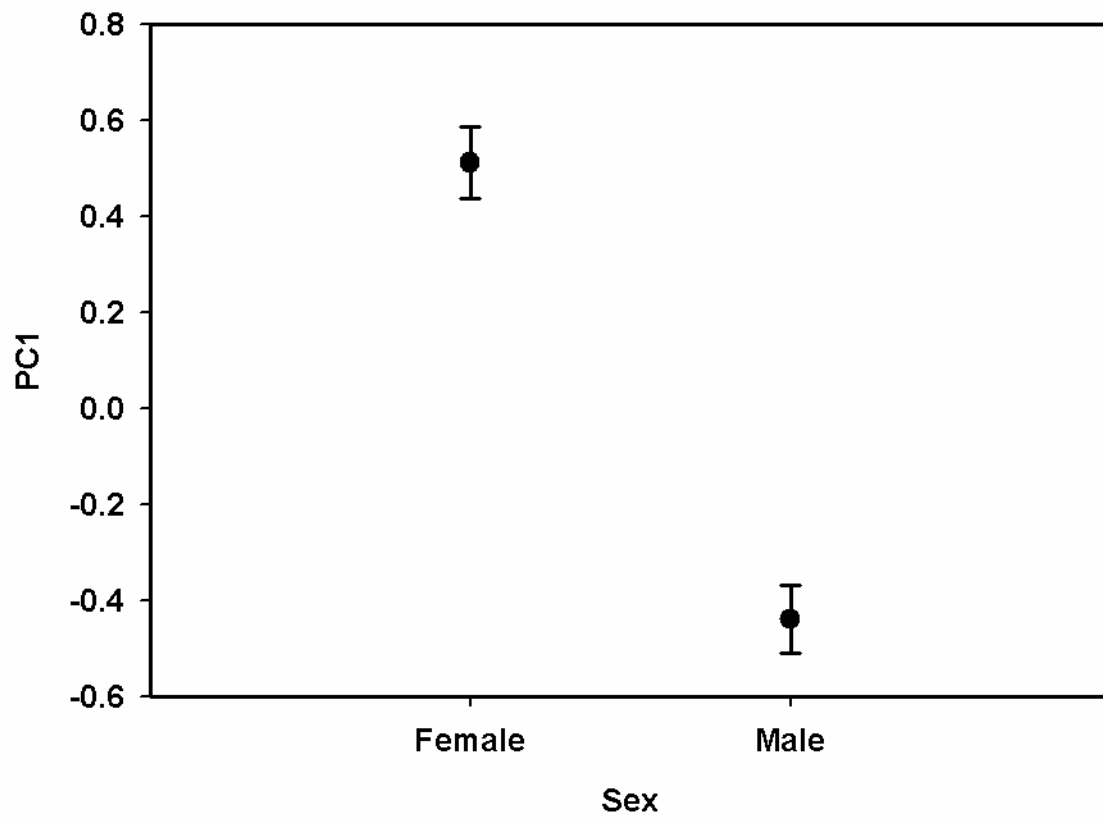


Figure 3

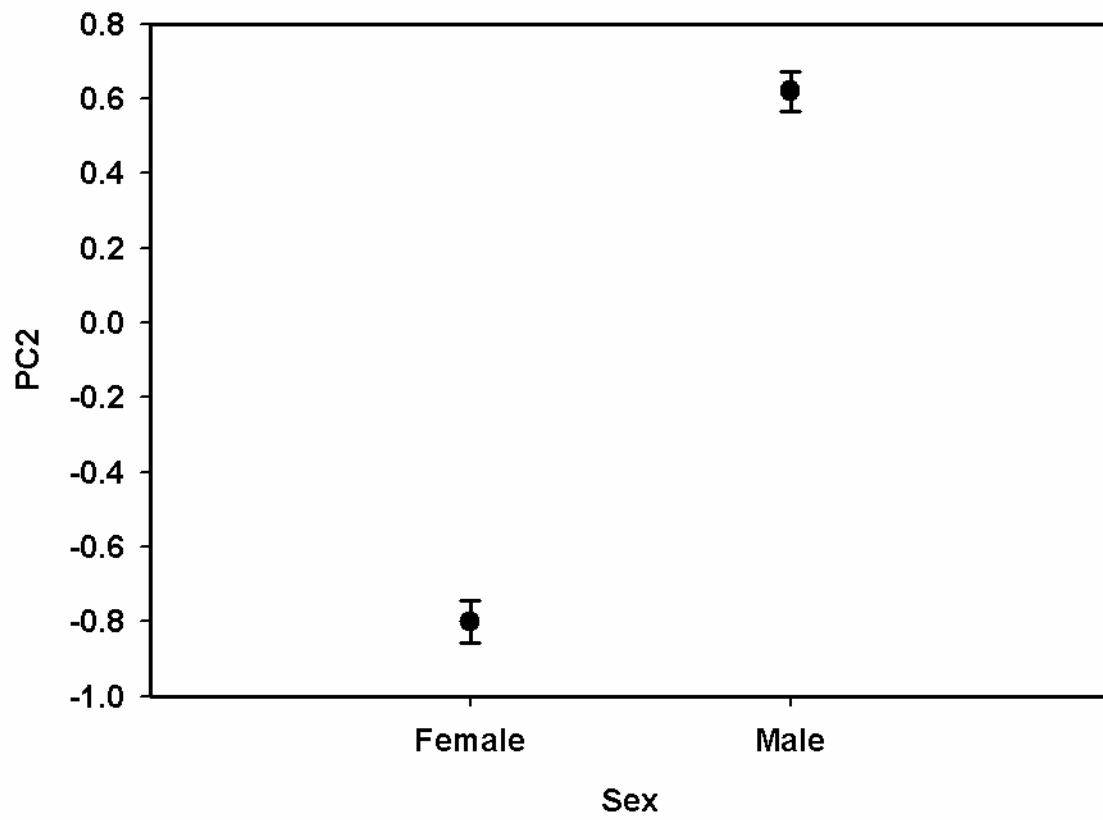


Figure 4A

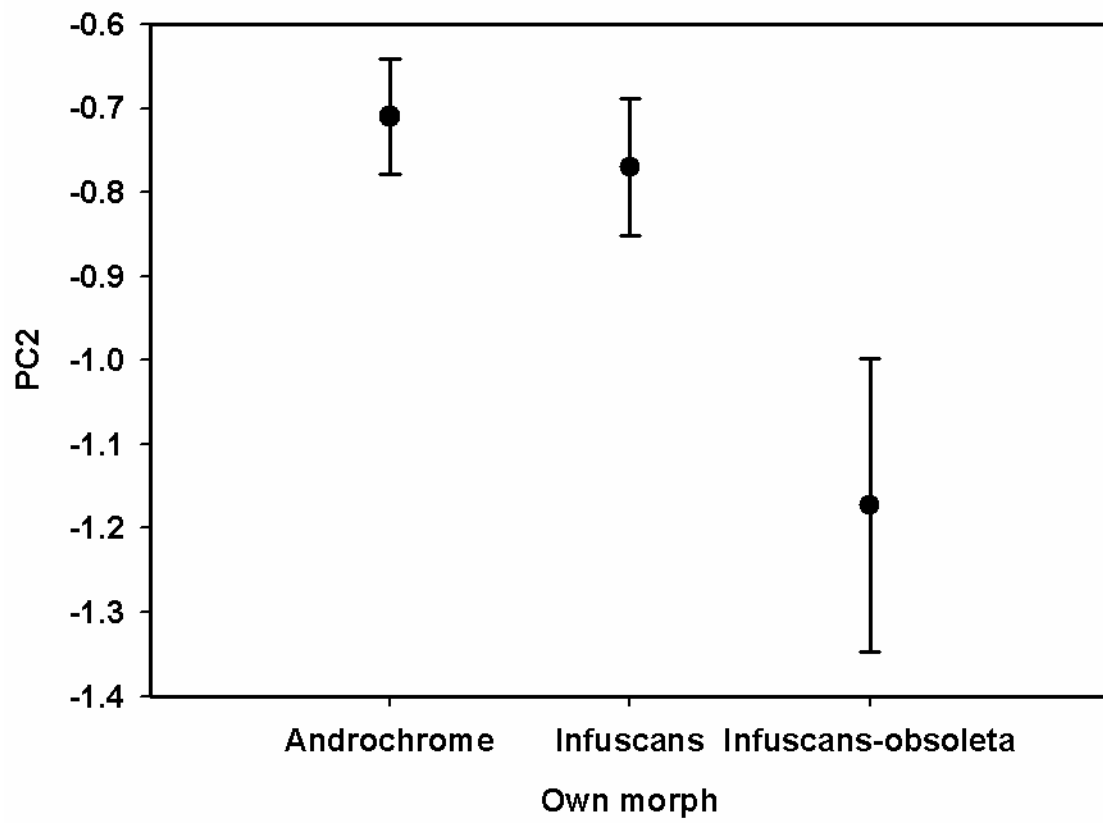


Figure 4B