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Convergence and divergence of the adaptive landscape in two ecologically similar and sympatric damselfly species
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28
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30   **Abstract**
31
32   Following the development of regression-based methods to estimate natural and sexual selection,
33   evolutionary biologists have quantified the strength, mode and direction of selection in natural
34   populations. Although this approach has been successful, its limitations include lack of
35   replication across species, compromising the generality of the inferences beyond
36   microevolutionary time scales. Here, we carried out a comparative selection study on wing shape
37   and body size, two important fitness-related traits, in two closely related and ecologically similar
38   pond damselflies: *Enallagma cyathigerum* and *Ischnura elegans* (family Coenagrionidae).
39   Through field surveys, we demonstrated that female *I. elegans* experience stronger male mating
40   harassment while the opportunity for sexual selection is greater in males of *E. cyathigerum*
41   compared to *I. elegans*. Our data revealed directional selection favoring large body size in
42   females of both species, weak stabilizing selection on wing shape in both sexes of both species,
43   while curvilinear sexual selection on male body size was divergent between the two species. Our
44   study thus revealed both convergence and divergence of the adaptive landscape, and illustrates
45   that even closely related sympatric species can differ substantially in their selective regimes due
46   to differences in their social organization and mating systems.
47
48   **Key words**: Adaptive landscape, natural selection, sexual selection, *Ischnura elegans*,
49   *Enallagma cyathigerum*
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Introduction

Since Russel Lande and Steven Arnold introduced regression-based statistical methods to quantify natural and sexual selection nearly four decades ago (Lande & Arnold, 1983), evolutionary biologists have sought to identify causes and consequences of phenotypic selection in nature (Endler, 1986; Mitchell‐Olds & Shaw, 1987; Wade & Kalisz, 1990; Kingsolver & Diamond, 2011). This has resulted in a rich scientific literature about the strength, mode and direction of selection in wild populations of animals and plants (Kingsolver *et al.*, 2001; Siepielski *et al.*, 2009, 2011, 2013, 2017; Kingsolver & Diamond, 2011). However, methodological problems remain and criticisms were directed towards naïve applications of these statistical methods. Early criticisms emphasized that trait-fitness covariances are not necessarily causal and should be viewed as preliminary hypotheses in need of experimental verification through trait manipulations (Mitchell‐Olds & Shaw, 1987), for instance, because of confounding factors such as environmental covariances between traits and fitness (Rausher, 1992; Stinchcombe *et al.*, 2002). Another important challenge is to identify environmental agents, demographic factors and ecological causes behind observed selection (Wade & Kalisz, 1990; Svensson & Sinervo, 2000; MacColl, 2011; Siepielski *et al.*, 2017). Different approaches have been suggested to verify the causality of selection, including path analysis (Kingsolver & Schemske, 1991), experimental manipulations of phenotypic traits (Sinervo *et al.*, 1992) or selective environments (Wade & Kalisz, 1990) or simultaneous "double-level" manipulations of both traits and selective agents (Sinervo & Basolo, 1996; Svensson & Sinervo, 2000). More recently, various issues associated with conceptualizations and measurements of selection have been identified, such as standardization of traits (Hereford *et al.*, 2004) and fitness (De Lisle & Svensson, 2017), measurement error in traits (Morrissey & Hadfield, 2012; Dingemanse *et al.*, 2021) and fitness (Waller & Svensson, 2016), and visualization of univariate (Schluter, 1988; Brodie *et al.*, 1995) and multivariate selection (Phillips & Arnold, 1989; Schluter & Nychka, 1994; Blows & Brooks, 2003; Blows *et al.*, 2004; Blows, 2007; Chenoweth *et al.*, 2012; Svensson *et al.*, 2021).

One way to address these problems is to combine information gathered from several different sources and integrate such information in a common conceptual framework. The adaptive landscape provides such a framework to unify phenotypic selection within populations with patterns of phenotypic divergence between populations and species (Arnold *et al.*, 2001; Svensson & Calsbeek, 2012). Under this framework, populations are conceptualized as evolving on a landscape which describes the relationship between relative fitness and phenotypic values, subject to selection, genetic drift and mutation (Lande, 1976). Populations are expected to climb the closest adaptive peak where short-term fitness is maximized (Wright, 1932) whereas genetic correlations can bias the evolutionary trajectories towards directions with high amount of additive genetic variance (Schluter, 1996; Walsh & Blows, 2009). Estimates of linear and quadratic selection coefficients and correlational selection (Phillips & Arnold, 1989; Brodie *et al.*, 1995; Svensson *et al.*, 2021) capture local landscape topography, which can in turn be used to infer the dynamics of landscape itself (Chevin & Lande, 2015; De Villemereuil *et al.*, 2020) and enable macroevolutionary predictions (Estes & Arnold, 2007; Uyeda *et al.*, 2011; Hansen, 2014). However, selection estimates do not in themselves inform us about ecological causes and selective agents. To infer selective agents and ecological causes of selection we also need to quantify local selective environments through functional analysis, experimental manipulations of

98 agents or collect detailed field observations in natural settings (Svensson & Sinervo, 2000;
99 MacColl, 2011; Opedal, 2021). In some cases, it is possible to directly identify selective causes
100 (Brown & Brown, 2013), e.g., by comparing surviving phenotypes with phenotypes killed by
101 predators (Young *et al.*, 2004; Svensson *et al.*, 2007; Kuchta & Svensson, 2014) or those
102 phenotypes dying from natural causes (Bumpus, 1899).

103

104 An underutilized approach is to compare closely related species inhabiting similar physical
105 environments and which largely share the same ecological niche. Comparative studies of
106 selection in different populations and/or between closely related species are still relatively few,
107 however, largely due to the logistical challenges to obtain sufficient spatial and temporal
108 replication (Nishida, 1994; Gosden & Svensson, 2008; Siepielski *et al.*, 2009, 2017; Punzalan *et*
109 *al.*, 2010; De Villemereuil *et al.*, 2020). The insect order Odonata (dragonflies and damselflies)
110 provides unique opportunities for such comparative studies of selection, because a rich
111 background knowledge of natural history (Corbet, 1999) enables researchers to characterize the
112 adaptive landscape in different environmental contexts. Here, we characterized the strength,
113 mode and direction of selection in two ecologically and phenotypically similar sympatric
114 damselfly species: the common bluetail damselfly (*Ischnura elegans*) and the common bluet
115 (*Enallagma cyathigerum*). These two species and other members of the family Coenagrionidae
116 (pond damselflies) have similar ecological niches and show evidence of neutral community
117 dynamics and ecological drift (McPeek & Brown, 2000; Siepielski *et al.*, 2010). Yet, recent
118 molecular evidence suggest that these two genera have been separated by >12 million years of
119 independent evolution (Swaegers *et al.*, 2014; Callahan & McPeek, 2016; Blow *et al.*, 2021). By
120 comparing phenotypic selection on homologous phenotypic traits in these close relatives that
121 inhabit similar environments, we addressed questions about stability and species differences in
122 the adaptive landscape and how landscape structure might influence phenotypic evolution at
123 macroevolutionary time scales.

124

125 We estimated univariate and multivariate selection on two important fitness-related traits in
126 sympatric populations of these species: body size and wing shape. Body size affects both male
127 mating success and female fecundity in many species of insects (Bonduriansky, 2001) and it is
128 often target of natural selection on fecundity in females and sexual selection in males (McCauley
129 & Wade, 1978; Arnqvist, 1992; Sokolovska *et al.*, 2000; Waller & Svensson, 2017). Mate choice
130 in damselflies is also affected by the fit between male (cerci) and female (mesostigmal plates)
131 reproductive structures, both of which are traits that scale with body size (McPeek *et al.*, 2008;
132 Steele *et al.*, 2011). Wing shape, in turn, is related to the flight performance in insects (Ellington,
133 1984; Dickinson *et al.*, 1999; Templin, 2000), and influences foraging efficiency, mate search
134 and predation avoidance, that constitute major determinants of adult fitness in damselflies
135 (Anholt, 1991).

136

137 We have three objectives. First, we compared demographic and mating system parameters of
138 these two species, including the opportunity for sexual selection (Arnold & Wade, 1984), mating
139 frequencies, male and female densities, operational sex ratio (OSR) and the frequency of male-
140 mimicking female color morphs ("androchromes"). These mating system parameters provide
141 information about the social system in these two species, including the role of sexual selection

142   through male-male competition (opportunity for sexual selection, OSR, male density) and the
143   intensity of male mating harassment (mating frequencies and the frequency of androchrome
144   female morphs as proxy for sexual conflict; see Svensson *et al.*, 2005; Gosden & Svensson,
145   2009; Takahashi *et al.*, 2014; Blow *et al.*, 2021). Second, we compared the adaptive landscapes
146   for wing shape and body size in both these species and in both sexes. Finally, we combined
147   information from these two sources to interpret the ecological and social causes of selection
148   explaining similarities and differences in the selective regimes. We also discuss the
149   macroevolutionary consequences of divergence and convergence in adaptive landscapes across
150   these species.

151

152   **Materials & Methods**

153

154   **Study system**: We study two species of damselflies, the common bluet, *Enallagma cyathigerum*
155   and the common bluetail damselfly, *Ischnura elegans* in the family Coenagrionidae (Odonata;
156   Zygoptera). These species are generalist predators that feed on small flying insects and they
157   occur in diverse aquatic habitats, typically open landscapes in ponds, lake shores, and slow
158   flowing streams (Smallshire & Swash, 2020). Both species are broadly distributed across the
159   Palearctic (Smallshire & Swash, 2020). Females of both species are polymorphic; females of *E.*
160   *cyathigerum* exhibit two color morphs: androchrome (mature females with blue thorax
161   mimicking males; Fig. 1A *ii*) and gynochrome (mature females with olive-green thorax; Fig. 1A
162   *iii*), while females of *I. elegans* exhibit three color morphs during the adult stage: androchrome
163   (mature females with blue thorax; Fig. 1A *v*), *infuscans* (Fig. 1A *vi*) and *infuscans-obsoleta* (Fig.
164   1A *vii*, Svensson *et al.*, 2020; Willink *et al.*, 2020). In southern Sweden, populations of *E.*
165   *cyathigerum* and *I. elegans* widely occur in sympatry. The reproductive season for both species
166   ranges from late April to early August. Males of both species engage in scramble competition
167   over the opportunity for mating. Males are non-territorial and found resting or flying near the
168   waterbody, chase any females that approach the waterbody, and compete for the opportunity to
169   grab the females by the prothorax using the clasper in the tip of males' abdomen. A male that
170   successfully clasps a female will form the "tandem position", followed by the formation of "a
171   mating wheel" (Fig. 1D) during which egg fertilization takes place (Corbet, 1999). After a
172   successful mating, male *E. cyathigerum* continues to guard the female in the tandem position
173   while the female oviposition over open water, whereas male *I. elegans* leave the female to
174   oviposition unguarded (M. Tsuboi, H. Frietsch and E.I. Svensson, personal observations; see also
175   Fig. 1E).

176

177   **Field work**: Field surveys were carried out to collect *I. elegans*, and *E. cyathigerum* at 20
178   different field sites in and around Lund, Sweden. Field sampling was conducted at small ponds
179   in the following localities: Borgeby, Bunkeflostrand, Flackarp, Flyinge 30 A1, Flyinge 30 A3,
180   Genarp, Gunnesbo, Hoje A14, Hoje A6, Hoje A7, Habo Gard, IKEA, Ilstorp, Krutladan,
181   Ladugårdsmarken, Lomma, Lunnarp, Råbydammen, Vombs vattenverk, and Vombs Bruksgård
182   (Fig. S1). Individuals were captured using hand-held nets while slowly walking around
183   waterbodies. Upon capture of individuals flying without a partner, we examined sex and kept
184   males and females separately in small net cages. Individuals found as either a tandem or as a
185   copulating couple were kept in plastic cups. We visited these populations between the hour of
186   08.00 and 13.00 in all partially or fully sunny days with temperature >15°C in May, June and

July of 2020 and 2021. At each visit, we sampled between 20 and 30 minutes, and between 3-5 people participated in the sampling. The captured damselflies were kept in cooling bags to protect them from overheating, and were brought back to the lab for recording phenotypic data and setting up mated females for egg-laying for fecundity measurements. For each individual, we recorded sex, female morph, and morphometric measurements (see next section for more detail). The dataset obtained from this field survey include 58 sampling visits to capture *I. elegans* (in 2020 and 2021) and 27 sampling visits to capture *E. cyathigerum* (in 2021), and constitutes in total of 497 single males, 236 single females, and 524 couples of *I. elegans* and 420 single males, 181 single females, and 411 couples of *E. cyathigerum*. This dataset will be used to estimate selection gradients presented further in this study.

In a complementary field study, we quantified and compare social organization and mating systems of *I. elegans* and *E. cyathigerum*. This was part of a community survey where we captured individuals of damselflies and dragonflies regardless of the species identity along predefined transects, with the aim of quantifying species composition, operational sex-ratio and density of local Odonata fauna. At each visit, 3-5 people sampled between 10-20 minutes, who identified and recorded all captured individuals in terms of species, sex, female color morph, sexual maturity and mating status (either captured as single or copula/tandem). After recording this information, animals were released. We used data of *I. elegans* and *E. cyathigerum* from four seasons (2018-2021) that were collected during a total of 366 visits. These data constitute a total observation of 3527 single males, 1788 single females, and 630 couples of *I. elegans* and 3031 single males, 403 single females, and 282 couples of *E. cyathigerum*. This dataset was used to compare and quantify differences in mating system and social organizations between these two species.

Fitness components: To measure phenotypic selection, we quantified two fitness components: (1) mating success that characterizes sexual selection in males and (2) fecundity that characterizes natural selection in females. Sexual selection on males was estimated by comparing the phenotypes of mated males with unmated males upon capture in the field. Males that were captured in tandem or in a copula were classified as 'mated' and assigned a mating success of '1', while males that were captured solitary were classified as 'single' and their mating success were assigned to '0'. This is an established technique of quantifying mating success in damselflies (Gosden & Svensson, 2008; Steele *et al.*, 2011). To measure female fecundity, we placed females captured in mated couples in small plastic cups with moist coffee filter to let the female lay eggs for 48hrs. The number of eggs laid by each female was subsequently counted. The number of eggs laid during this time interval likely reflects the recent ecological conditions a given female has experienced preceding oviposition, and should capture her past history of food intake rates and ambient temperatures (Svensson & Abbott, 2005; Svensson *et al.*, 2020). This fecundity measure thus provides a measure of fitness and natural selection in females.

Morphometric measurements and wing image acquisition: We obtained digital images of all captured individuals using a scanner (CanoScan 5600F) at a resolution of 600 dpi to get estimates of body size. Five linear measurements were taken for each individual to capture different aspects of the body size: total body length, thorax width, abdomen length, wing length, and width of the S4 segment (Fig. 1B). Individuals were measured from these photographs using the computer program, Fiji (Schindelin *et al.*, 2012). All measurements were originally recorded

232   in units of pixel, which was then converted to millimeter (mm) based on a conversion between
233   mm and pixel at a resolution of 600 dpi. After the scan has been completed, individuals were
234   sacrificed by exposing them to cold temperature. From sacrificed individuals, we dissected fore-
235   and hindwings from both left and right side of the body. Dissected wings were then placed in a
236   wet coffee filter, covered with a transparent plastic sheet, then scanned at a resolution of 2000
237   dpi. We obtained images for a total of 5764 wings belonging to *E. cyathigerum*, and a total of
238   7704 wings belonging to *I. elegans*.

239

240   **Automated assay of wing shape using ML-morph**: We used a recently developed machine
241   learning tool "ML-morph" (Porto & Voje, 2020) to measure the x-y coordinates of 17 landmark
242   that characterize wing venation patterns (Fig. 1C). First, a training set was constructed to train
243   ML-morph to identify the landmark positions in the wing image. We used a training set
244   containing 400 wing images that was landmarked manually using Imglab version 1.18 (compiled
245   from Python module dlib-19.23). These 400 images subset in the training set contained a random
246   mixture of 100 images of the 4 wings (right forewing, right hindwing, left forewing, left
247   hindwing) of a male *I. elegans*, 100 images of a female *I. elegans*, 100 images of a male *E.*
248   *cyathigerum*, and 100 images of a female *E. cyathigerum*. This training set represents variation
249   in wing shape with respect to sex, species, and fore- or hind-wing assignment, that are
250   considered as main source of variation in our sample set.

251   We used *shape.predictor* function of the ML-Morph to train the algorithm for landmarking the
252   wing. Then, we tested the ability of ML-Morph to landmark wings accurately using *shape.tester*
253   function, where we compared automated landmarking by ML-morph to manual landmarks,
254   which responded to 99.2% precision in the landmarking using automation in a test set build from
255   the training set. Finally, we applied *shape.predictor* function to landmark the remaining image
256   set that have not been landmarked. All landmarked images were later checked manually for any
257   errors, and inconsistent landmarks across all images were corrected. We removed 357 images
258   (178 for *I. elegans* and 179 for *E. cyathigerum*) from the dataset before analysis because they
259   contained an injured/broken wing where position of 1 or more landmarks could not be deduced
260   accurately. Examination of erroneous images were done by one observer (AG).

261

262   **Geometric morphometrics and dimension reduction**: The wings of the two damselfly species
263   were standardized through geometrics morphometrics analysis. We performed a Generalized
264   Procrustes Analysis (GPA) on the 17 landmarks including all measured wings using geomorph
265   package version 4.0.5. GPA transforms the landmarks by rotating, aligning, and scaling so that
266   the resulting landmarks (i.e., aligned coordinates) describe the wing shape alone. The aligned
267   coordinates, however, still have 30 dimensions, and dimensional reduction was necessary for
268   further analyses. We employed two complementary approaches for dimension reduction. First,
269   we performed a principal component analysis (PCA) on the matrix containing the aligned
270   coordinates of both species to reduce dimensionality of the major axis of morphological
271   variation. Second, we performed a linear discriminant analysis (LDA) on the aligned coordinates
272   with species as a classifier using MASS package version 7.3-53. We also performed a principal
273   components analysis (PCA) on the covariance matrix of natural logarithmic values of the five
274   size traits (total body length, thorax width, abdomen length, wing length, and width of the S4
275   segment) for both species and sexes. We log-transformed the values for the size traits before

running the PCA, so that the resultant PC values became mean-standardized. We averaged values obtained from left and right side of the wing of an individual whenever both sides were measured. When only one side of the wing was available, we used that available side as a representative measure. Since we found no directional asymmetry in wing shape in two species we examined (results not shown), this will unlikely create systematic bias in our measurements. In 62 individuals, we obtained scan images twice at two independent scanning sessions to evaluate measurement error due to variation in our imaging process and found that the measurement errors in aligned coordinates associated with the difference in images are on average 0.19% of the centroid size (range: 0% - 3.34%, Fig. S2). Since this level of error is negligeable compared to the effect sizes of all analyses presented in this research, it will not be accounted for in subsequent statistical analyses of wing shape.

Quantifying social organization and mating system: For quantitative estimation of social organization in the two damselfly species, we measured the following parameters: male density (total number of males caught per sampling event / total catching time, unit = number of individuals captured per minute), female density (total number of males caught per sampling event / total catching time, unit = number of individuals captured per minute), operational sex ratio (ratio of the number of mature males to the number of mature females of a species caught at a locale in a given year, unit = %), androchrome frequency (ratio of the number of the androchrome female morph of a species to the total number of females of a species whose morph was identified caught in one locale in one year, unit = %), the proportion copulating males (number of males in copula / total number of males caught during one sampling, unit = %) and the opportunity for sexual selection (I_s, variance in male mating success / (average male mating success)^2, unitless elasticity) (Arnold & Wade, 1984). We estimated I_s for every locale from where we sampled populations of *E. cyathigerum* and *I. elegans*. Male mating success was defined as a binomial variable that could be either 0 or 1. We calculated variance in male mating success based on the data on the proportion of single and copulating males at each site and mean male mating success. The latter (mean male mating success) was calculated by the mean proportion of male (belonging to one species) mating at a particular locale in one field season (e.g., 0.10 if 10% are found in copula). The variance in mating success was evaluated as $\frac{p \times (1-p)}{n}$ where p is the probability of being found as a couple and n is the sample size. We tested for the difference in social organization indices between *E. cyathigerum* and *I. elegans* using a mixed effect model implemented in lme4 package (Bates *et al.*, 2015) with one of the six parameters as the response variable, species as the fixed explanatory variable and sampling year and location at which individuals were captured (hereafter referred to as locale) as the random effect.

Selection gradients: We used standard multiple-regression analyses to estimate the selection gradients (Lande & Arnold, 1983). Selection gradients were evaluated for body size (size-PC1) and wing shape (LD1) using mating success in the field as a male fitness component and the number of eggs laid as the female fitness component. Both linear (β) and quadratic (γ) selection coefficients were estimated for both species and sexes for all the traits using linear mixed effect models with locale as the random factor. The partial regression coefficients of models that includes only linear (i.e. unsquared) term were used to estimate β, while γ was estimated as the partial regression coefficients of models that includes both unsquared and squared terms. The

319 quadratic regression coefficients were multiplied by two (Stinchcombe *et al.*, 2008). We also
320 performed full multivariate analysis on both these traits to visually verify the quadratic
321 relationships using cubic splines (Schluter, 1988). Fitness components (mating success for male
322 and the number of eggs laid for females) were standardized within species and sexes, by dividing
323 individual fitness with the mean fitness estimates (De Lisle and Svensson 2017). Since both size-
324 PC1 and wing shape LD1 are scaled to its own value either by taking a natural logarithm of size
325 measurements or by performing the Generalized Procrustes Analyses, selection gradients
326 evaluated in these traits are on the mean-standardized scale without additional transformations.
327 For comparison, we also estimated the variance-standardized selection gradients by dividing
328 them with standard deviation of the trait within species.

329 Estimation of selection gradients and statistical comparisons of differences between species were
330 made using random mixed effect models. We constructed three sets of models, which all
331 included locale as the random effect. First, for each sex and species separately, we modeled
332 fitness component as the response variable and one of examined traits (size-PC1, forewing shape
333 LD1, hindwing shape LD1) and its squared terms as the fixed explanatory variables. Second, we
334 constructed two bivariate models, for each sex and species separately, with the same model
335 specifications as above but include either size-PC1 and forewing shape LD1 or size-PC1 and
336 hindwing shape LD1 and their interaction and squared terms as the fixed explanatory variables.
337 Finally, we constructed two models for each sex separately, with the fitness component as the
338 response variable, size-PC1 and forewing shape LD1 or size-PC1 and hindwing shape LD1 with
339 their squared terms, species (either *I. elegans* or *E. cyathigerum*) and interaction terms between
340 species and all traits as the fixed explanatory variables. In this model, we identified the best
341 model with a backward model selection procedure based on AICc (sample size-corrected Akaike
342 Information Criterion) values. In this approach, a comparison of AICc between two nested
343 models with and without a focal parameter provides statistical support for the effect of the focal
344 parameter. All analyses were performed in RStudio version 4.0.4 and visualization of data and
345 results were performed using ggplot2 package version 4.0.5.

346

347 **Results**

348

349 **Social organization and mating system:** Comparison of social organization between *I. elegans*
350 and *E. cyathigerum* are presented in Figure 2. We found statistical evidence for differences in
351 social organization and mating system between the two species in all examined parameters
352 except for male density (Table S1). Males of *I. elegans* were roughly twice as more often found
353 as mating couple as *E. cyathigerum* (proportion of copula; *I. elegans*: mean ± SE = 0.129 ±
354 0.015, *E. cyathigerum*: 0.077 ± 0.013), experienced 39% less opportunity of sexual selection (I_s)
355 than *E. cyathigerum* (I_s; *I. elegans*: mean ± SE = 0.211 ± 0.044, *E. cyathigerum*: 0.345 ± 0.052),
356 and females of *I. elegans* had twice as high frequency of the male-mimicking phenotype
357 (*androchrome*) as *E. cyathigerum* (androchrome frequency; *I. elegans*: mean ± SE = 0.458 ±
358 0.046, *E. cyathigerum*: 0.267 ± 0.116). The operational sex-ratio is male-biased in both species,
359 but significantly more so in *E. cyathigerum*, which partly reflects low overall density of females
360 near the pond in this species compared to *I. elegans*.

361

362 **Dimension reduction of body size and wing shape:** A scatterplot of the first two principal

363 components of five body size measurements (Fig. S3) revealed clear and statistically significant
364 size differences between *I. elegans* and *E. cyathigerum* and between sexes in both species (Fig.
365 3A, Table S2). The degree of sexual dimorphism in body size is more pronounced in *I. elegans*
366 than in *E. cyathigerum,* as revealed by significant interaction term between species and sex
367 (estimate ± SE = -0.277 ± 0.009, p < 0.001, Table S2). Since the first PC axis explained the vast
368 majority (93.0 %) of the total variation in log of body size measurements, we hereafter used this
369 axis (size-PC1) as our measure of body size.

370 Wing shape differed between *I. elegans* and *E. cyathigerum* and between fore- and hindwings in
371 both species (Fig. S4). The first principal component (PC1) explained 41.9% of the total
372 variation, and this axis separates forewings from hindwings in terms of the width of the wing.
373 The forewings of both species have lower PC1 values along PC1 axis, which can be interpreted
374 as narrower wings, while the hindwings of both species have higher values of PC, translating to
375 broader wings. The second principal component (PC2) explained 19.2% of the total variation in
376 wing shape, and this axis separates the two species. *E. cyathigerum* has broader proximal edge
377 and narrower distal edge of wings as compared to *I. elegans*. Subsequent PC axes up to PC5
378 explained 8.9%, 6.3%, and 5.9% of total variation, respectively.

379 A linear discriminant function analysis (LDA) with species as a grouping factor revealed that the
380 axis that most effectively separates wings of the two species (wing shape LD1) represents the
381 variation in the width of the wing, that occurs together with the stretch of two landmarks (LM9
382 and LM16) at the center of wing (Fig. 3B). We found statistical support for sexual dimorphism in
383 wing shape except for forewings of *E. cyathigerum* (Table S3) but visual inspection of effect
384 sizes revealed that these sex differences in wing shape are small relative to sexual dimorphism in
385 body size (Fig. 3). In what follows, we focus on LD1 because the goal of this study is to compare
386 to *I. elegans* and *E. cyathigerum* with respect to overall wing shape, and LD1 captures this
387 variation most efficiently. Results based on the first five principal components of wing shape are
388 presented in supplementary materials.

389

390 **Selection gradients**: Selection gradients of size PC1 are presented in Table 1. Across both
391 species, we found that selection favors females with large body sizes. Estimates of mean-
392 standardized directional selection gradients (β) in females (*I. elegans*: β ± SE = 1.569 ± 0.295, *E.*
393 *cyathigerum*: 4.138 ± 0.545) means that doubling of body size would increase relative fitness by
394 157% in *I. elegans* and 414% in *E. cyathigerum*. In males, selection on size PC1 was weaker,
395 whereas the quadratic selection gradients (γ) in *E. cyathigerum* indicate disruptive selection
396 (γ±SE = 5.551 ± 2.726) in favor of either small or large individuals.

397 Selection on wing shape LD1 was relatively weak across both sexes in the two species (Fig. 4,
398 Table 1). The directional selection gradients (β) showed that the selection typically favors
399 narrow and elongated wings in *I. elegans* while broad and round wings are favored in *E.*
400 *cyathigerum*. Considering the mean shape differences between the two species (Fig. 3B), these
401 estimates suggest that, if all else being equal, directional selection would drive the convergent
402 evolution of wing shape. The quadratic selection gradients (γ) suggested stabilizing selection
403 (i.e., negative γ) on wing shape LD1 in all trait-sex-species combination except for forewing of
404 *E. cyathigerum,* although not all these negative estimates were significant (Table 1). Together
405 with the result of β that favors intermediate wing shape of the two species, wing shape appear to
406 be under weak stabilizing selection in both *I. elegans* and *E. cyathigerum*. For both size PC1 and

407 wing shape LD1, variance-standardized gradients showed qualitatively equivalent results (Table
408 1). Results based on PC1 to PC5 of wing shape are presented in supplementary materials (Table
409 S4, Fig. S5).

410 The multivariate selection gradients, including correlational selection between size PC1 and
411 shape LD1, are presented in Table 2, Figure 5, and Figure S6. Estimates of gradients are
412 generally consistent with those obtained from univariate models, with one notable exception. In
413 males of *E. cyathigerum*, the evidence for disruptive selection on body size in the univariate
414 estimate of γ is shown to result from a combination of directional selection favoring small size (β
415 $\pm$ SE = -5.335 $\pm$ 2.104), a non-significant trend of disruptive selection on wing shape ($\gamma \pm$ SE =
416 8.632 $\pm$ 5.592, p = 0.12), and a marginal effect of negative correlational selection between body
417 size and wing shape ($\gamma \pm$ SE = -1.224 $\pm$ 0.661, p = 0.06). Visualization of this selection surface
418 revealed a complex adaptive topography in males of *E. cyathigerum* characterized by a fitness
419 saddle with two fitness peaks where large and small males had higher mating success (Fig. 5B).
420 The fitness surface of males of *I. elegans* was flatter with a tendency for weak stabilizing
421 selection on body size ($\gamma \pm$ SE = -3.272 $\pm$ 2.234, p = 0.07). By contrast, the adaptive landscapes
422 of females were simpler than males in both species. Correlational selection was virtually absent
423 and fitness monotonically increased with body size consistent with the univariate estimates.

424

425 **Comparison of adaptive landscape between species**: Comparisons of the adaptive landscape
426 between *I. elegans* and *E. cyathigerum* are summarized in Table S5. Pair-wise comparisons
427 between a model and its nested model based on AICc values indicate that the adaptive landscape
428 is divergent between the two species in terms of the quadratic terms of body size in males
429 (ΔAICc = 6.6 in a model with forewing, ΔAICc = 7.3 in a model with hindwing) and in terms of
430 the linear term of body size in females (ΔAICc = 7.8 in a model with forewing, ΔAICc = 8.3 in a
431 model with hindwing). Thus, the disruptive selection on male body size in *E. cyathigerum* is
432 absent or even takes the form of stabilizing selection in *I. elegans* (Table1, Fig. 4A) while in
433 females, directional selection favoring large body sizes is stronger in *E. cyathigerum* than in *I.
434 elegans* (Table1, Fig. 4D)

435

436 **Discussion**

437

438 ***Ischnura elegans* and *Enallagma cyathigerum* differ in their social organization**

439

440 Comparisons of mating system parameters in *I. elegans* and *E. cyathigerum* revealed interesting
441 differences (Fig. 2). Compared to *E. cyathigerum*, *I. elegans* have higher female densities (Fig.
442 2B), higher frequency of androchrome females (Fig. 2D), higher mating rates (Fig. 2E) and the
443 opportunity for sexual selection is lower in this species (Fig. 2F). These species differences
444 suggest that the form of sexual selection differs between *I. elegans* and *E. cyathigerum*. In *E.
445 cyathigerum*, there is a classical mating system based on male-male competition over the
446 opportunity for mating. In contrast, in *I. elegans*, sexual conflict through male mating harassment
447 of females is the primary mechanism of sexual selection in *I. elegans* (Fig. 2C). Thus, although
448 these species largely share the same habitat and engage in similar scramble male mate
449 competition over females, the mechanism of sexual selection appear to differ. An important

450   qualitative difference between the mating system of these species might be differences in
451   postcopulatory mate guarding during female oviposition (Corbet, 1999). In *I. elegans*, females
452   oviposit alone, unguarded by the male, where they are likely to be more susceptible to mating
453   harassment from other males who try to mate with vulnerable females during their oviposition
454   phase. In contrast, in *E. cyathigerum*, males remain attached to the female in tandem position,
455   even during female oviposition, suggesting that females are less vulnerable to male mating
456   harassment (M. Tsuboi, H. Frietsch and E.I. Svensson, personal observations; Fig. 1E). These
457   subtle but fundamental differences in pre- and post-mating behavior are likely causes of mating
458   system differences and social structure between these two ecologically similar species.

459

460   **Causes of divergence and convergence in body size and wing morphology**

461

462   With this ecological background information of these two species in mind, we compared wing
463   shape and body size, two traits that are targets of selection in adult Odonata (Sokolovska *et al.*,
464   2000; Steele *et al.*, 2011; Outomuro *et al.*, 2016), between *I. elegans* and *E. cyathigerum* with the
465   goal to understand the ecological causes behind the selective regimes operating on these traits.
466   We found that *I. elegans* exhibit a more pronounced female-biased sexual size dimorphism than
467   *E. cyathigerum* (Fig. 2A; Table S2). This might suggest difference in the form of sexual selection
468   between *I. elegans* and *E. cyathigerum* has resulted in species difference in the degree of sexual
469   size dimorphism. In *I. elegans*, small-size could be advantageous to maneuver in dense
470   vegetation where mating typically take place. This preferred microhabitat in *I. elegans* might
471   have driven down body size in males of this species. Previous work on sexual selection in *I.*
472   *elegans* have revealed variable sexual selection on male body size, which can favor small males,
473   large males or intermediate-sized males depending on ecological context and geographic position
474   (Gosden & Svensson, 2008; Dudaniec *et al.*, 2022). In contrast, maneuverability might be less
475   important in mate competition among male *E. cyathigerum*, where mating predominantly takes
476   place over the open water and where the male remain attached to the female for an extended
477   period of time during oviposition (Fig. 1E).

478   An alternative explanation for the difference in sexual size dimorphism between these two
479   species is that natural or sexual selection in *I. elegans* females might have increased female body
480   size in this species. For instance, stronger sexual conflict in *I. elegans* might have selected for
481   larger females that can defend themselves against mate-harassing males. We also demonstrated
482   that there is positive fecundity-selection towards larger female size in both these species (Fig.
483   4D). Without additional and independent knowledge about the ancestral states in male and
484   female body sizes of these two species, we cannot say which of these two historical scenarios is
485   true. Nevertheless, the findings here should stimulate further investigation of body size evolution
486   in a broader macroevolutionary context involving more species in these two genera (Swaegers *et*
487   *al.*, 2014; Callahan & McPeek, 2016; Blow *et al.*, 2021).

488   Although body size was strongly sexually dimorphic in *I. elegans*, we did not find as pronounced
489   sexual dimorphism in wing shape in neither this species nor *E. cyathigerum* (Fig. 3B). However,
490   there was a clear overall species differences in wing shape, with *E. cyathigerum* having longer
491   and more pointed wings compared to the shorter and more rounded wings in *I. elegans*. These
492   species differences in wing shape might reflect different microhabitat use and mating system
493   differences. As explained above, *I. elegans* mainly mate in the vegetation around ponds and

494 exhibits no postcopulatory mate guarding, *E. cyathigerum* mate more in the open areas and males
495 remain attached to females during oviposition and when copulating pairs fly over water bodies
496 (M. Tsuboi, H. Frietsch and E. Svensson, unpublished observations). More elongated wings in *E.*
497 *cyathigerum* might reflect these more open habitat preferences, whereas the shorter and more
498 rounded wings of *I. elegans* might instead reflect that mating largely takes place in vegetation
499 and that males and females do not remain attached during oviposition with no postcopulatory
500 mate guarding. Consistent with this interpretation, ecomorphological comparative studies of
501 birds indicate that habitat structure is an important determinant of wing shape evolution, with
502 species in more dense habitats having shorter and more rounded wings and species occupying
503 more open habitats having longer and more pointed wings (Kennedy *et al.*, 2016). Our
504 knowledge about the functional relationship between flight performance and wing shape in
505 Odonata is more limited (Ellington, 1984; Bomphrey *et al.*, 2016) but if we assume that similar
506 aerodynamic and ecological principles apply to damselfly wings as bird wings (Kennedy *et al.*,
507 2016), these species differences in wing shape are broadly consistent with species differences in
508 their microhabitats. Thus, the more open-dwelling *E. cyathigerum* has longer and narrower
509 wings and the more vegetation-dwelling *I. elegans* having rounder and broader wings.

510

511 **Causes and consequences of divergence and convergence in adaptive landscape**

512

513 Females in both species experienced positive directional selection towards large body size
514 although this fecundity selection is stronger in magnitude in *E. cyathigerum* than in *I. elegans*
515 (Fig. 4; Table 1; Table 2; Table S5). Thus, the fitness benefits in terms of fecundity increased
516 faster with female size in *E. cyathigerum* than in *I. elegans*. Intense male mating harassment in
517 females of *I. elegans* compared to *E. cyathigerum* can potentially explain these species
518 differences and this divergent selection on females, since male mating harassment in *I. elegans*
519 has been shown to be mainly directed towards high-fecundity females (Gosden & Svensson,
520 2009). In other insects such as *Drosophila*, large females have intrinsically higher fecundity but
521 also suffer more from male mating attempts reducing this fitness advantage (Long *et al.*, 2009;
522 Chenoweth *et al.*, 2015). In contrast, in *E. cyathigerum,* female fecundity benefits of large size
523 might be less affected by male mating harassment that seems to be lower in this species
524 compared to *I. elegans* based on several mating system indices. Lower proportion of
525 androchrome females in *E. cyathigerum* than in *I. elegans* is particularly important here, as
526 androchrome females in damselflies benefit from reduced male mating harassment as shown in
527 many previous studies in the genus *Ischnura* (Robertson, 1985; Cordero *et al.*, 1998; Gosden &
528 Svensson, 2009; Willink *et al.*, 2019; Blow *et al.*, 2021). Hence, a lower frequency of
529 androchrome females in *E. cyathigerum* than in *I. elegans* is likely to reflect relaxed mating
530 harassment and reduced sexual conflict in the former species. In addition, female *E. cyathigerum*
531 are locally segregated from males of the same species, as previously documented in several other
532 species of damselflies (Stoks, 2001; Steele *et al.*, 2011), whereas in *I. elegans*, males and females
533 typically occur in sympatry, and sex ratios are more even in this species (Fig. 2C). Sexual habitat
534 segregation might therefore further release females of *E. cyathigerum* from the cost of male
535 mating harassment, enabling them to enjoy greater fecundity advantages of large body size
536 compared to *I. elegans*. Taken together, female body size in *E. cyathigerum* and *I. elegans* is
537 subject to directional selection towards larger size and this fecundity selection is significantly
538 stronger in the former species.

539 Compared to females, selection on male body size was erratic. We found divergent patterns of
540 selection between the two species where *I. elegans* is characterized by a weak (non-significant)
541 stabilizing selection while *E. cyathigerum* is under a disruptive selection. However, when
542 correlational selection between wing shape and body size were considered, there was instead an
543 indication of negative directional selection towards small body size in *E. cyathigerum*. We
544 interpret these results as indicating that sexual selection on male body size is highly context
545 dependent in these pond damselflies. Previous studies have shown that the form and magnitude
546 of sexual selection in damselflies can vary in a fine spatial and temporal scale depending on local
547 community structure, such as density of female color morph (Gosden & Svensson, 2008) or
548 phenotypic distribution of male and female body sizes (Steele *et al.*, 2011).

549 Selection on wing shape in these two species differed somewhat depending on if fore- or
550 hindwing were considered, but in general it was stabilizing (Fig. 4). All three significant
551 quadratic selection estimates were negative in sign and six out of the total eight estimates were
552 negative (Fig. 4; Table 2). This indicates that wing shape is under stabilizing selection both in
553 terms of sexual selection in males and fecundity selection in females. One interpretation is that
554 wing shape is currently maintained around some optimum shared by both sexes within a species
555 but with the two species occupying different adaptive peaks. An alternative interpretation is that
556 these species occupy the same peak of a macroevolutionary adaptive landscape in which the two
557 species resides at different locations of the peak depending on their phenotypic values. Our
558 results are in favor of the latter scenario. Selection estimates revealed that, among eight estimates
559 of directional selection gradient (β) we evaluated, β of *E. cyathigerum* were all positive in sign
560 and β of *I. elegans* were all negative in sign (Table 1). Based on the difference in wing shape
561 between *E. cyathigerum* and *I. elegans* (Fig. 2), these estimates can be interpreted as convergent
562 selection towards an intermediate wing shape of the two species. It is conceivable that the
563 intermediate wing phenotype represents a global adaptive optimum that characterizes
564 fundamental mechanical and physiological demands of flight, that would constitute the primary
565 optimum (*sensu* Hansen, 1997), within which each species occupy a subset of this global optima
566 depending on species-specific ecological niches that collectively form the adaptive zone (*sensu*
567 Simpson, 1944).

568 That selection on wing shape was largely stabilizing in both species and thus convergent
569 contrasts markedly with selection on body size that was directional in females and weaker but
570 more variable in males. One implication of the different modes of selection on these two traits is
571 that we would expect wing shape to evolve more slowly over macroevolutionary time scales
572 compared to body size. Our data support this expectation because body size is clearly different
573 between *I. elegans* and *E. cyathigerum* while the difference in wing shape between the two
574 species is much modest, which is in fact smaller than the difference between fore- and hindwing
575 of a single individual (Fig. 3, Fig. S4). More generally, wing morphology of insects, particularly
576 that of Dipterans has been put forward as an example of macroevolutionary stasis (Hansen &
577 Houle, 2004; Houle *et al.*, 2017). Our findings are thus consistent with the idea that the
578 macroevolutionary stasis reflects the stability of macroevolutionary adaptive landscape (Estes &
579 Arnold, 2007; Uyeda *et al.*, 2011). We further note that females of *E. cyathigerum* experience
580 stronger directional selection towards large body size than females of *I. elegans*, and *E.*
581 *cyathigerum* also happens to be the larger species of the two (Fig. 3A, Fig. S2). Thus, the current
582 species difference in size is the same as would be expected if such directional selections have
583 persisted over macroevolutionary time scales. These findings suggest that selection gradients

584   measured at contemporary populations may have an implication for macroevolutionary
585   phenomena, at least when compared species share large fraction of their ecological niches.

586

587   **Conclusions**

588

589   This comparative selection study of two phenotypically and ecologically similar damselfly
590   species have revealed both shared and unique aspects of the adaptive landscapes of two key
591   traits: body size and wing shape. Broadly speaking, body size is subject to stronger and more
592   variable selection than wing shape. We interpret this as partly reflecting sex-specific selection
593   but also a result of species differences in mating systems and different degree of sexual conflict
594   through male mating harassment on females. In contrast, wing shape seems largely to experience
595   a similar mode of stabilizing selection in both species that is concordant between the sexes and
596   may result in convergent evolution in wing shape of the two species. This may indicate closely
597   located fitness optima of these two species reside within an adaptive zone of a shared
598   macroevolutionary adaptive landscape that has been stable for millions of years. These species-
599   specific optima of wing shape are likely to reflect structural and physical differences in the
600   microhabitats of these two species. We suggest that both these physical microenvironmental
601   differences but also social and mating system differences between these two phenotypically
602   similar species can explain some of the differences in the adaptive landscapes in these two
603   ecologically similar species. These damselfly species and most other organisms are therefore not
604   solely passive objects of selection, but they also shape the selection pressures on themselves
605   (Lewontin, 1983). Results in this study suggest that some aspects of the selection on phenotypic
606   traits in these two ecologically similar species arise from intrinsic mating system differences,
607   which could be a potential example of social niche construction (Saltz *et al.*, 2016) and more
608   broadly illustrate the reciprocal interactions and feedbacks between organisms and their
609   environments (Odling-Smee *et al.*, 2003).

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611

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856

857 Table 1: Mean-standardized linear (β) and quadratic (γ) selection gradients for body size (PC1), and wing shape (LD1) to complement

858 Figure 4. Estimates within square brackets "[]" are variance-standardized selection gradients. Estimates that are statistically

859 significantly different from 0 at a significance level of $p < 0.05$ are shown in **bold**. All estimates are obtained from mixed-effect

860 models that include sampled location as a random effect.

Trait	Sex	β ± SE		γ ± SE	
		I. elegans	*E. cyathigerum*	*I. elegans*	*E. cyathigerum*
Body size PC1	♂	0.053±0.200 [0.008±0.032]	-0.036±0.388 [-0.003±0.036]	-3.234±2.026 [-0.082±0.052]	**5.551±2.726**[*] [**0.094±0.046**[*]]
	♀	**1.569±0.295**[***] [**0.222±0.042**[***]]	**4.138±0.545**[***] [**0.415±0.055**[***]]	-3.761±3.275 [-0.076±0.066]	-5.578±6.698 [-0.056±0.067]
Wing shape LD1 (forewing)	♂	-0.018±0.036 [-0.015±0.032]	0.080±0.057 [0.050±0.036]	**-0.057±0.027**[*] [**-0.064±0.025**[*]]	0.133±0.109 [0.049±0.046]
	♀	**-0.112±0.050**[*] [**-0.094±0.042**[*]]	0.137±0.091 [0.086±0.057]	**-0.090±0.041**[*] [-0.074±0.039]	0.145±0.145 [0.054±0.064]
Wing shape LD1 (hindwing)	♂	-0.051±0.036 [-0.045±0.032]	0.046±0.054 [0.030±0.036]	**-0.052±0.025**[*] [**-0.065±0.024**[**]]	-0.145±0.087 [-0.056±0.047]
	♀	-0.063±0.050 [-0.052±0.042]	**0.194±0.094**[*] [**0.118±0.057**[*]]	-0.071±0.036 [-0.064±0.036]	-0.137±0.186 [-0.059±0.074]

861
862 Note: * indicates $0.01 < p < 0.05$, ** indicates $0.001 < P < 0.01$, *** indicates $p < 0.001$.

863 Table 2: Mean-standardized linear (β) and quadratic (γ) selection gradients of body size (PC1), and wing shape (LD1) that

864 characterizes multivariate selection surfaces shown in Figure 5. Estimates that are statistically significantly different from 0 at a

865 significance level of $p < 0.05$ are shown in **bold**. All estimates are obtained from mixed-effect models that include sampled location as

866 a random effect.

Sex	Parameters	estimates ± SE (forewing)		estimates ± SE (hindwing)	
		I. elegans	*E. cyathigerum*	*I. elegans*	*E. cyathigerum*
♂	(intercept)	0.954±0.226	2.512±0.546	0.964±0.239	1.292±0.544
	Wing shape LD1	0.015±0.083	0.618±0.318	-0.005±0.079	-0.223±0.277
	Wing shape LD1^2	-0.067±0.037	0.089±0.112	-0.052±0.033	-0.159±0.088
	Body size PC1	-0.861±0.907	**-5.335±2.104$^{(*)}$**	-1.069±0.944	**-4.476±2.119$^{(*)}$**
	Body size PC1^2	-3.272±2.234	8.632±5.592	-3.146±2.130	10.056±5.482
	LD1 × PC1	-0.172±0.285	-1.224±0.661	-0.071±0.268	-0.740±0.619
♀	(intercept)	1.069±0.117	-0.377±0.998	0.963±0.129	-0.442±0.929
	Wing shape LD1	0.101±0.082	0.492±0.468	0.144±0.078	0.245±0.509
	Wing shape LD1^2	**-0.096±0.048$^{(*)}$**	0.166±0.140	**-0.079±0.040$^{(*)}$**	-0.015±0.184
	Body size PC1	**2.109±0.883$^{(*)}$**	**7.006±3.077$^{(*)}$**	**2.482±0.907$^{(**)}$**	**6.212±2.980$^{(*)}$**
	Body size PC1^2	-3.995±3.704	-4.892±6.815	-4.759±3.558	-5.791±6.846
	LD1 × PC1	-0.381±0.436	0.408±0.867	-0.469±0.401	-0.036±0.875

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868 Note: * indicates $0.01 < p < 0.05$, ** indicates $0.001 < P < 0.01$, *** indicates $p < 0.001$.
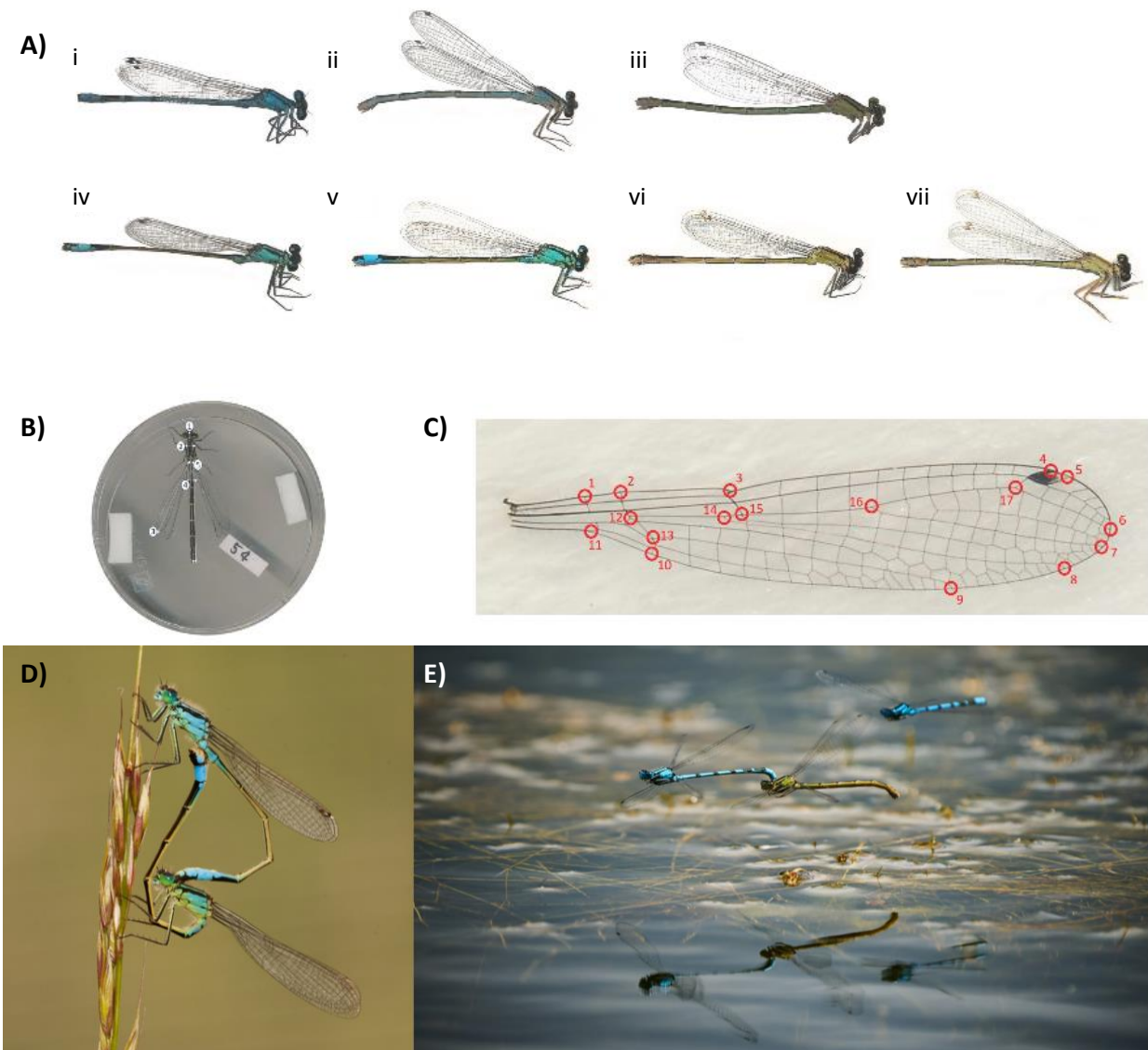
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Main Figures:

Figure 1: A) Males and female phenotypes and different color morphs in *E. cyathigerum* (i to iii) and *I. elegans* (iv to vii). Each subpanel shows an image of the following phenotype: males (i, iv), androchrome female (ii, v), gynochrome female (iii), infuscans female (vi), *infuscans-obsoleta* female (vii). Note that in both *E. cyathigerum* and *I. elegans* there is a male-mimicking female morph (androchrome females) but in former species there is only one gynochrome color morph, whereas in the latter there are two (*infuscans* and *infuscans-obsoleta*, respectively). B) Morphometric measurements of a female *E. cyathigerum* (all other males and females of *E. cyathigerum* and *I. elegans* were also measured similarly); 1: body length, 2: thorax width, 3: wing length, 4: width of abdomen segment S4, and 5: total abdomen length; C) 17 landmark position on a left forewing of a male *E. cyathigerum* that were used for estimation of the wing shape (all other males and females of *E. cyathigerum* and *I. elegans* were also landmarked similarly). D) a mating pair of *I. elegans* forming a mating wheel. E) a pair of *E. cyathigerum* forming a tandem.
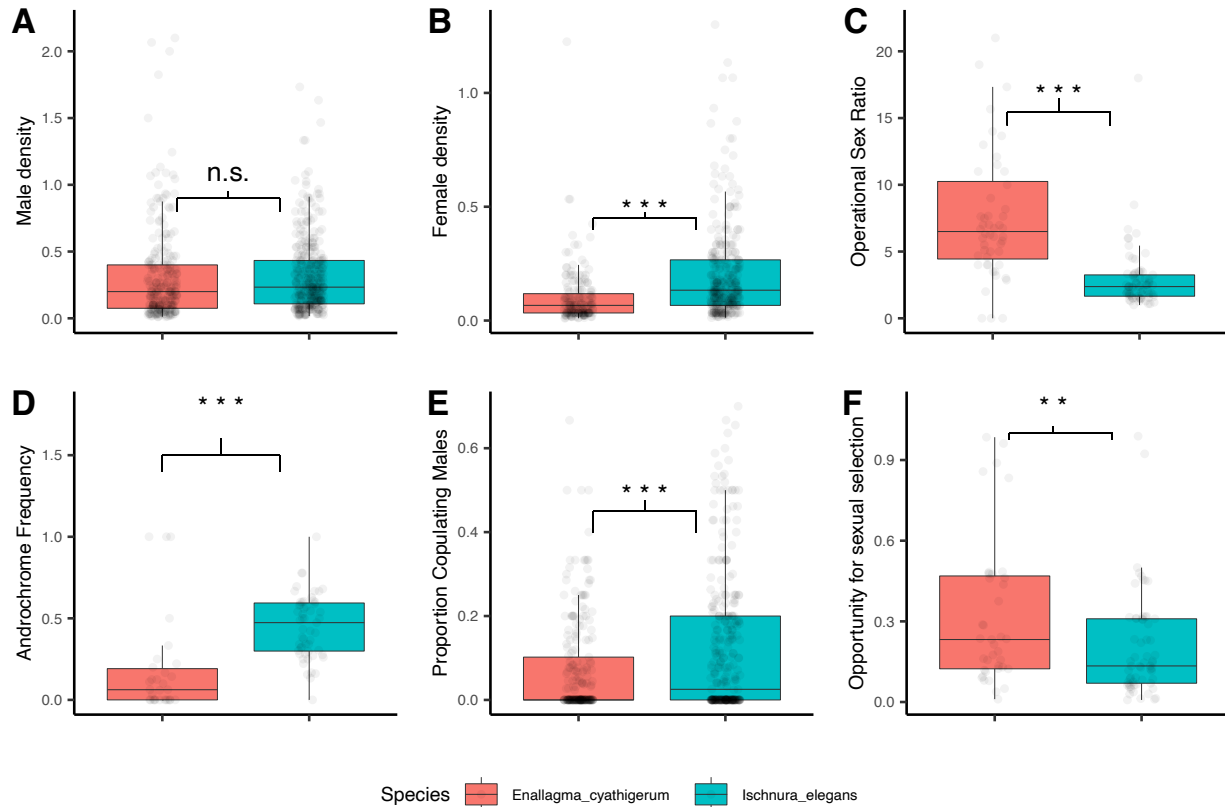
Figure 2: Boxplots (barline in the boxplot represent 50th percentile and the intervals represent the range between 1.5 times above 75th percentile and below 25th percentile) comparing demographic and mating system parameters of *E. cyathigerum* and *I. elegans* across four consecutive years (2018, 2019, 2020, 2021) of sampling. A) Male density, B) female density, C) operational sex ratio, D) androchrome frequency, E) proportion of copulating males, and F) opportunity for sexual selection. The estimates for the mean and standard errors of these parameters are as well as the statistical analysis for comparison between the two species is presented in Table S1 in the supplementary information. Symbols of significance: ***P < 0.001, **P < 0.01, *P<0.05.
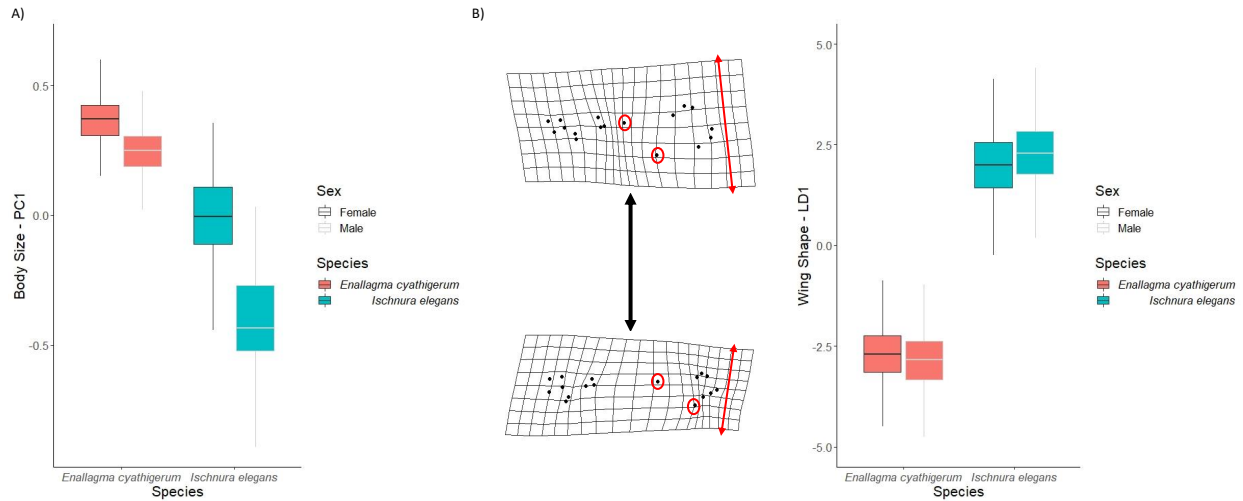
Figure 3: A) Boxplot of body size (PC1) difference between *E. cyathigerum* and *I. elegans* and for males and females within each species (statistical results are presented in Table S2); B) Boxplot representing variation in wing shape (LD1) across *I. elegans* and *E. cyathigerum.* The landmark specimens visualized in this figure were made by exaggerating the minimum and maximum LD1 values by a factor of two (statistical results are presented in Table S3).
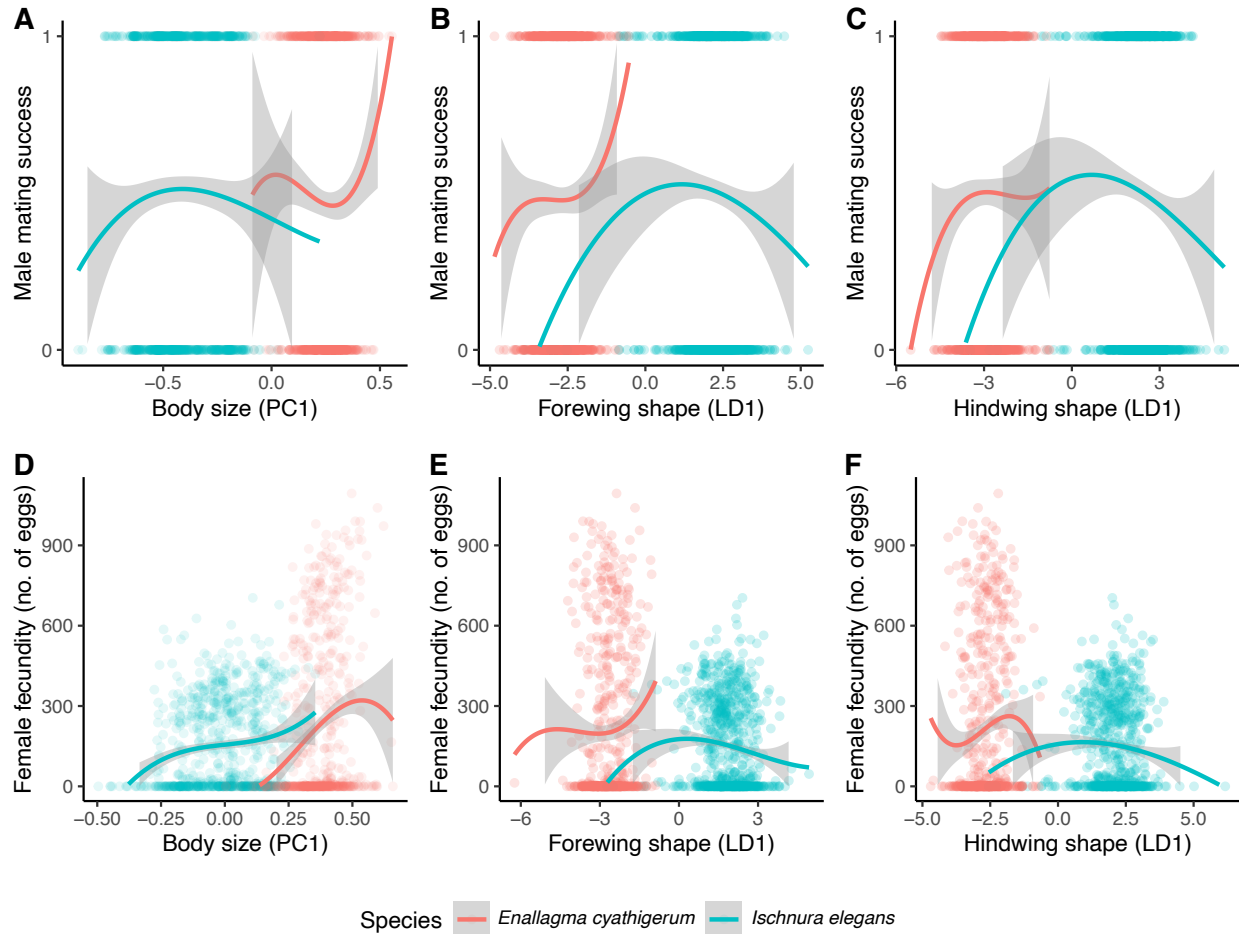
Figure 4: Univariate sexual selection (males, A-C) and fecundity selection (females, D-F) on body size (PC1) and wing shape (LD1) in *E. cyathigerum* and *I. elegans*. Fitness functions visualized using cubic splines and the "loess" function in R. Fitness data on the Y-axis show raw male mating success (1: mated, 0: unmated) and female fecundity (no. of eggs laid). Note that, in our estimations of sexual selection and fecundity-selection, fitness were relativized within species. The gray shaded regions around each spline represents the 95% confidence intervals. A) Selection on body size (PC1) in males, B) Selection on male forewing shape (LD1), C) Selection on male hindwing shape (LD1), D) Selection on body size (PC1) in females, E) Selection on female forewing shape (LD1) F) Selection on female forewing wing shape (LD1). The estimates for the coefficients of selection are presented in Table 1.
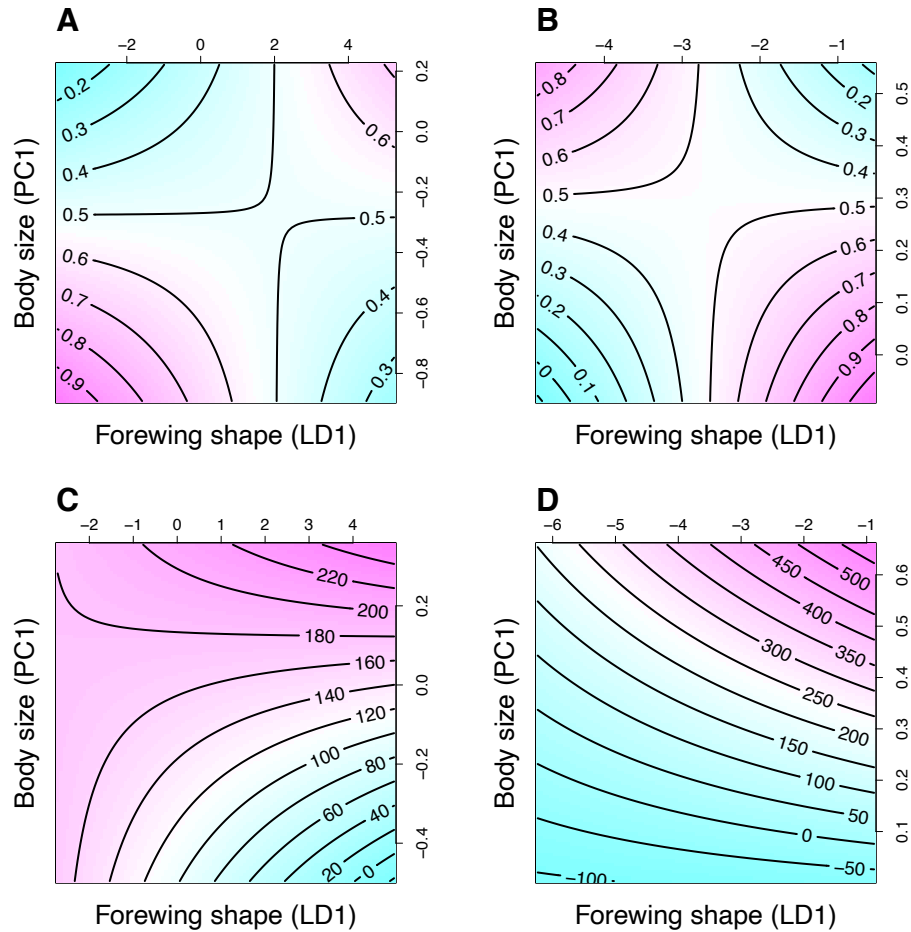
Figure 5: Bivariate fitness surfaces of forewing shape (LD1) and body size (PC1) in A) males of *I. elegans*, B) males of *E. cyathigerum*, C) females of *I. elegans* and D) females of *E. cyathigerum*. The axis perpendicular to the presented plane represents relative fitness, which is the predicted probability of being found as mated in male (A and B) and the predicted number of eggs laid by females (C and D). These fitness surfaces are based on splines from multivariate generalized additive models. The selection coefficients from this model are presented in Table 2. Bivariate fitness surfaces of hindwing shape (LD1) and body size (PC1) are presented in Figure S6.