Convergence and divergence of the adaptive landscape in two ecologically similar and sympatric damselfly species

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Abstract

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

Key words: Adaptive landscape, natural selection, sexual selection, *Ischnura elegans*, *Enallagma cyathigerum*

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

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

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

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

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

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

Materials & Methods

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

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

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

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

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

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

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

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

Results

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

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

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

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

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

Discussion

Ischnura elegans and Enallagma cyathigerum differ in their social organization

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

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

Causes of divergence and convergence in body size and wing morphology

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

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

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

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

Causes and consequences of divergence and convergence in adaptive landscape

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

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

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

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

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

Conclusions

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

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

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

Table 2: Mean-standardized linear (β) and quadratic (γ) selection gradients of body size (PC1), and wing shape (LD1) that characterizes multivariate selection surfaces shown in Figure 5. Estimates that are statistically significantly different from 0 at a significance level of $p < 0.05$ are shown in **bold**. All estimates are obtained from mixed-effect models that include sampled location as 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

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

Main Figures:

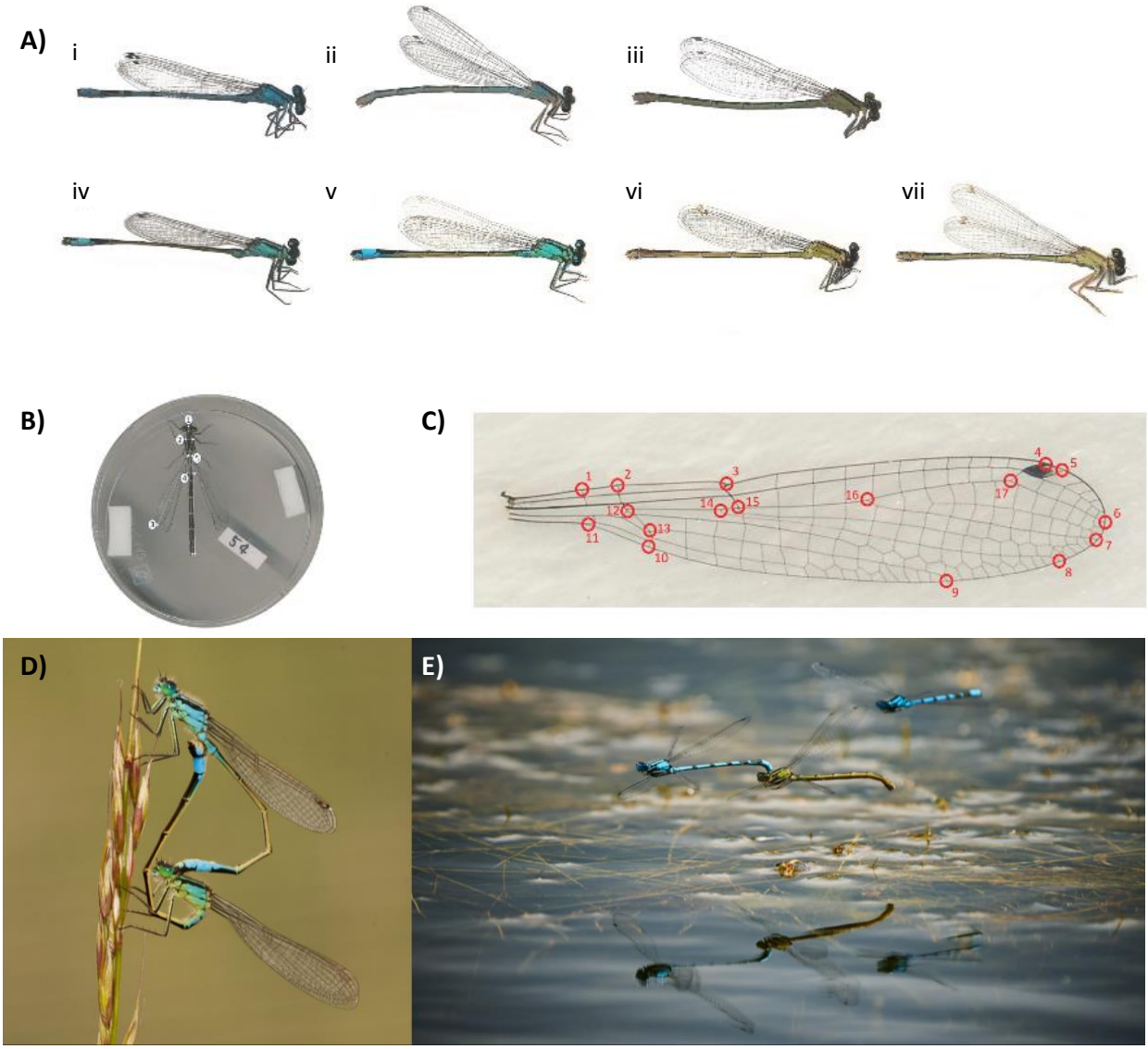

Figure 1: A) Males and female phenotypes and different color morphs in *E. cyathigerum* (i to iii) and *I. elegans* (iv to vii). Each subpanel shows an image of the following phenotype: males (i, iv), androchrome female (ii, v), gynochrome female (iii), infuscans female (vi), *infuscans-obsoleta* female (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.

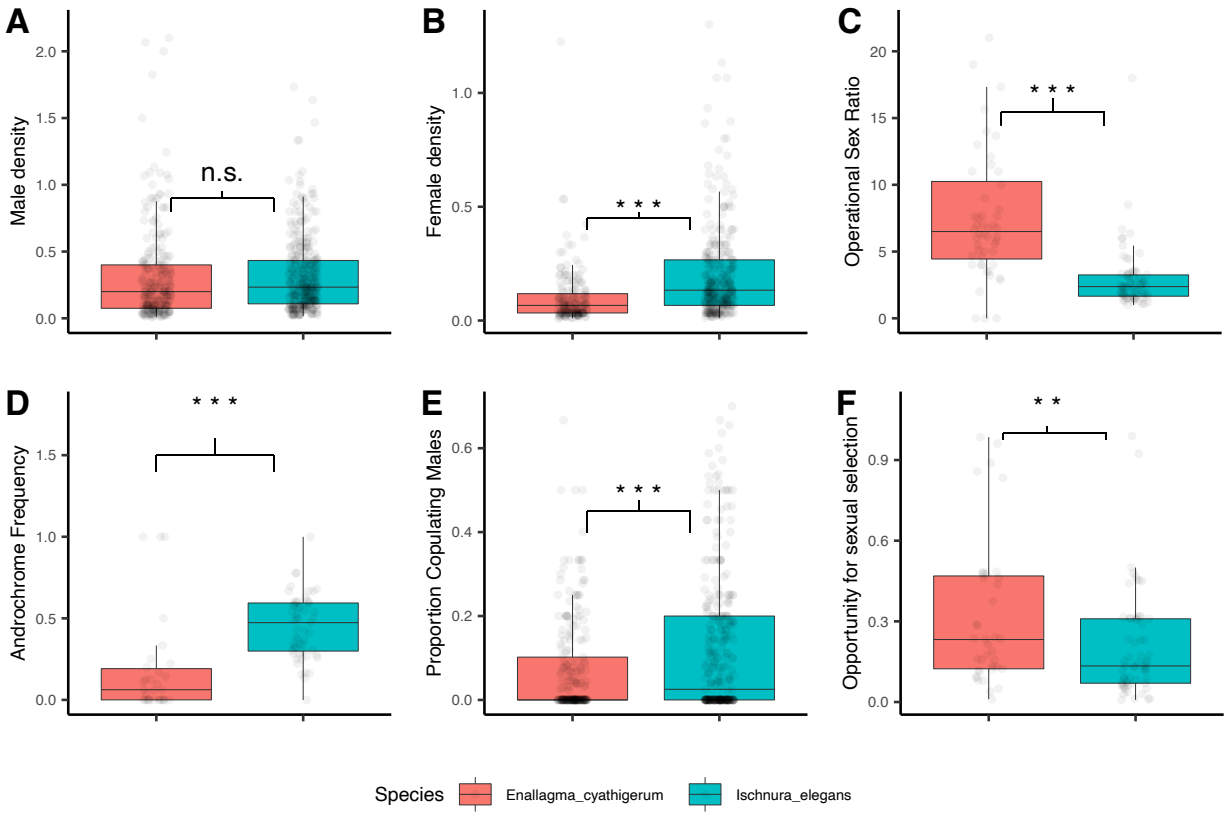

Figure 2: Boxplots (barline in the boxplot represent 50th percentile and the intervals represent the range between 1.5 times above 75th percentile and below 25th percentile) comparing demographic and mating system parameters of *E. cyathigerum* and *I. elegans* across four consecutive years (2018, 2019, 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.

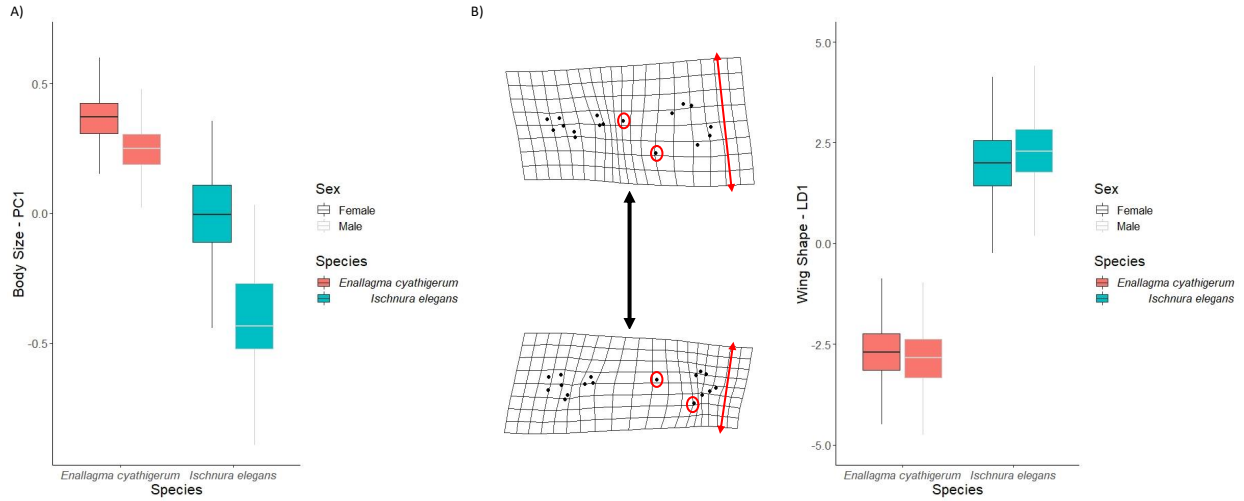

Figure 3: A) Boxplot of body size (PC1) difference between *E. cyathigerum* and *I. elegans* and for males and females within each species (statistical results are presented in Table S2); B) Boxplot representing variation in wing shape (LD1) across *I. elegans* and *E. cyathigerum.* The landmark specimens 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).

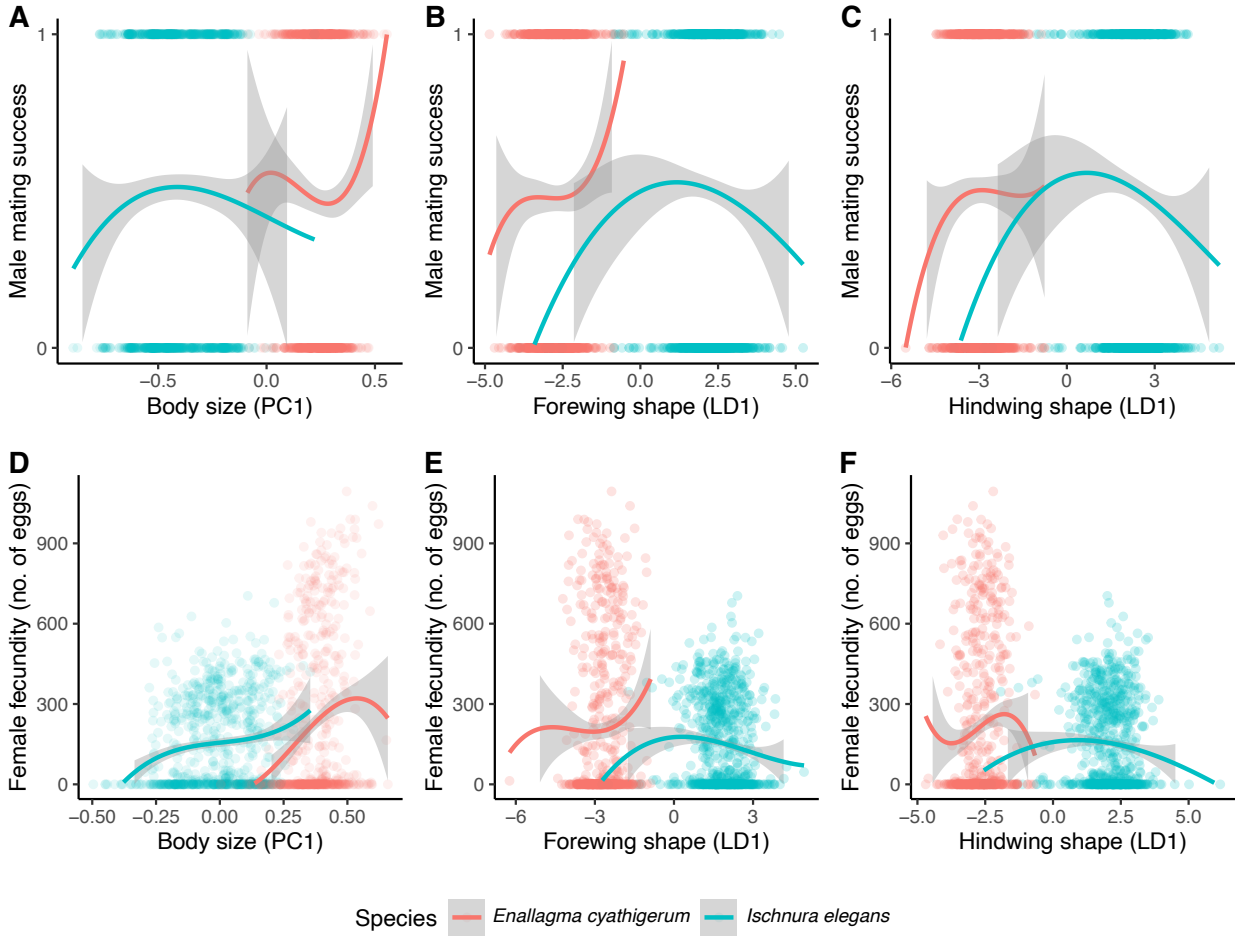

Figure 4: Univariate sexual selection (males, A-C) and fecundity selection (females, D-F) on body size (PC1) and wing shape (LD1) in *E. cyathigerum* and *I. elegans.* Fitness functions visualized using cubic splines and the "loess" function in R. Fitness data on the Y-axis show raw male mating success (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.

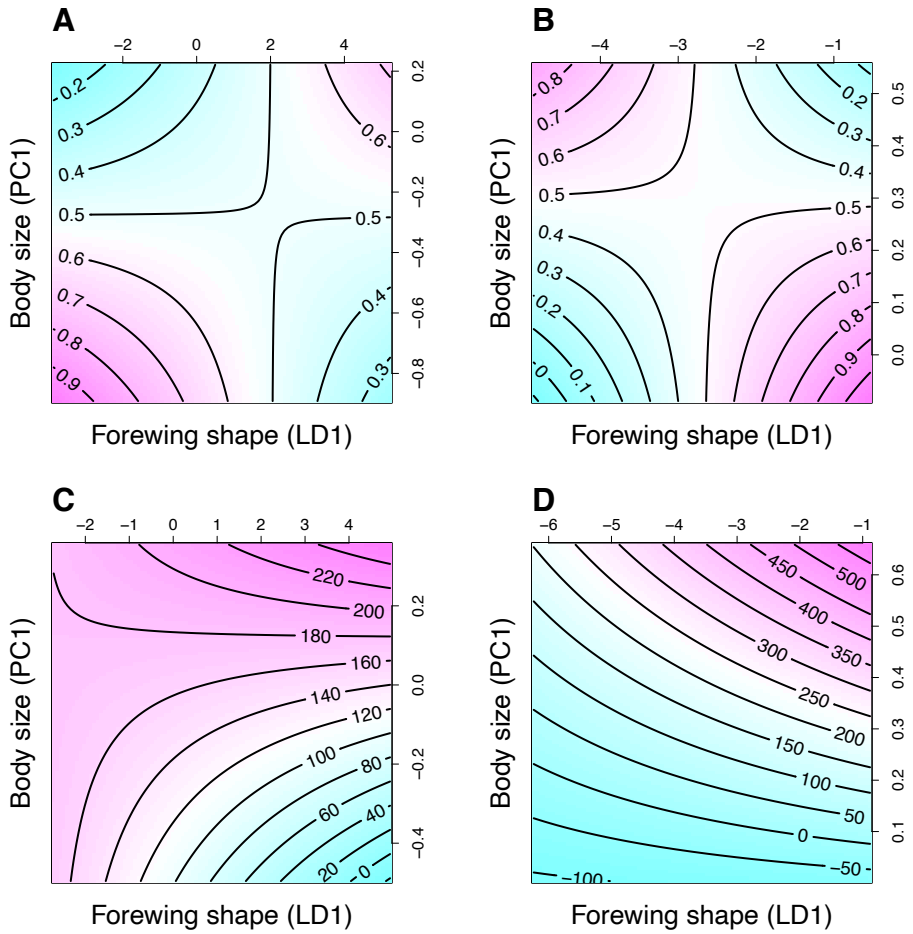

Figure 5: Bivariate fitness surfaces of forewing shape (LD1) and body size (PC1) in A) males of *I. elegans*, B) males of *E. cyathigerum*, C) females of *I. elegans* and D) females of *E. cyathigerum.* The axis perpendicular to the presented plane represents relative fitness, which is the predicted 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.