

Predator-Mediated Natural Selection on the Wings of the Damselfly *Calopteryx splendens*: Differences in Selection among Trait Types

Shawn R. Kuchta^{1,*} and Erik I. Svensson²

1. Department of Biological Sciences, Ohio University, Athens, Ohio 45701; 2. Evolutionary Ecology Unit, Biology Department, Lund University, SE223-62 Lund, Sweden

Submitted July 3, 2013; Accepted January 28, 2014; Electronically published May 15, 2014

Online enhancement: appendix. Dryad data: <http://dx.doi.org/10.5061/dryad.sf804>.

ABSTRACT: Traits that increase mating success in males may come at a cost, such as an increased risk of predation. However, predator-mediated selection is challenging to document in natural populations, hampering our understanding of the trade-offs between sexual selection and predation. Here we report on a study of predator-mediated natural selection on wing traits in the damselfly *Calopteryx splendens*, the males of which possess conspicuous wing patches. Wagtails (genus *Motacilla*) are important avian predators of *C. splendens*, capturing them in flight and removing the wings prior to consumption. Using geometric morphometric techniques, we quantified the strength and mode of selection on wing traits by comparing wings from depredated individuals with the standing variation present in the population. Our results reveal that predator-mediated selection is stronger on secondary sexual characters than on size and shape, suggesting that traits related to flight performance are closer to their adaptive peaks. This could be a consequence of the long-term evolutionary association with avian predators, whereas stronger selection on conspicuous secondary sexual traits may reflect trade-offs between sexual and natural selection. Finally, even though *C. splendens* possesses nearly identical fore- and hindwings, we found evidence for divergent selection between them.

Keywords: fitness landscape, geometric morphometrics, predation, secondary sexual trait, selection gradient, selective agent.

Introduction

Strong selection commonly stems from competition for mating opportunities, and sexual selection is the cause of much impressive morphological diversification (Michael et al. 1994; Simmons and Scheepers 1996; Schillaci 2006). However, while sexually selected ornaments and armaments improve mating success, they may also entail costs,

such as impaired locomotor performance (Allen and Levinton 2007), compromised immune function (Svensson et al. 2009), or an elevated risk of parasitism (Zuk and Koluru 1998), all of which may increase the risk of predation (Ryan et al. 1982; Godin and McDonough 2003). Predator-mediated natural selection should therefore check the evolutionary exaggeration of many secondary sexual characters. Unfortunately, the strength and mode of predator-mediated selection in natural populations have not been studied as much as other forms of selection (but see Brodie 1992; Young et al. 2004; Brown and Brown 2013; Cunningham et al. 2013). Predation is challenging to observe directly, making it difficult to positively identify the agents of selection that generate a correlation between traits and viability (MacColl 2011). Predation also removes individuals from a population, preventing their subsequent measurement. As a consequence, the majority of studies of predator-mediated selection are indirect, as they either demonstrate an evolutionary response to selection (Reznick et al. 1997), infer the historical role of predator-mediated evolution (Kuchta 2005; Kuchta et al. 2008; Eroukhmanoff and Svensson 2009), or investigate the macroevolutionary signatures of predator-prey interactions (Clapham and Karr 2012).

Here we report results from a study of predator-mediated natural selection in the damselfly *Calopteryx splendens* (banded demoiselle). This species presents a rare opportunity for quantifying the strength and mode of predator-mediated selection in the wild where the agent of selection is known. Wagtails (genus *Motacilla*) are important natural avian consumers of *C. splendens* (Svensson and Friberg 2007; Rantala et al. 2011). After catching damselflies, wagtails frequently return to “slaughter stations” (Svensson and Friberg 2007), where the wings are discarded prior to consumption. By comparing discarded wings recovered from slaughter stations

* Corresponding author; e-mail: kuchta@ohio.edu.

to the standing variation present in a population, the strength and mode of natural selection on wing traits can be estimated (Young et al. 2004; Rantala et al. 2011; Cunningham et al. 2013).

In calopterygid damselflies, wings function in more than flight performance, as the males of many species possess melanized color patches on their wings, often with an additional iridescence of green or blue (Svensson and Waller 2013). In *C. splendens*, the color patch fills roughly 50% of the wing area (fig. 1), though the extent of melanization varies within and among populations (Svensson et al. 2004; Tynkkynen et al. 2004). The size and opacity of wing patches in males play important roles in male-male competition, female choice, and species recognition (Plaistow et al. 1996; Siva-Jothy 1999, 2000; Cordoba-Aguilar 2002; Svensson et al. 2004, 2006; Tynk-

kynen et al. 2004, 2005, 2006; Toivanen et al. 2009; Rantala et al. 2010). Females do not have wing patches, but instead possess hyaline wings with a green tint.

In this article we focus on differences in predator-mediated natural selection on traits related to flight performance (wing size and shape) versus secondary sexual traits (wing color patch size and opacity). If wing size and shape are optimized for flight performance and thus sit near an adaptive peak with regard to aerial predator-prey interactions, one may expect to find evidence of stabilizing selection on these traits. To the contrary, meta-analyses of selection suggest that stabilizing selection is found considerably less often than expected (Kingsolver et al. 2001, 2012). One explanation for this counterintuitive finding is that stabilizing selection may be difficult to detect in natural populations even if it is ubiquitous

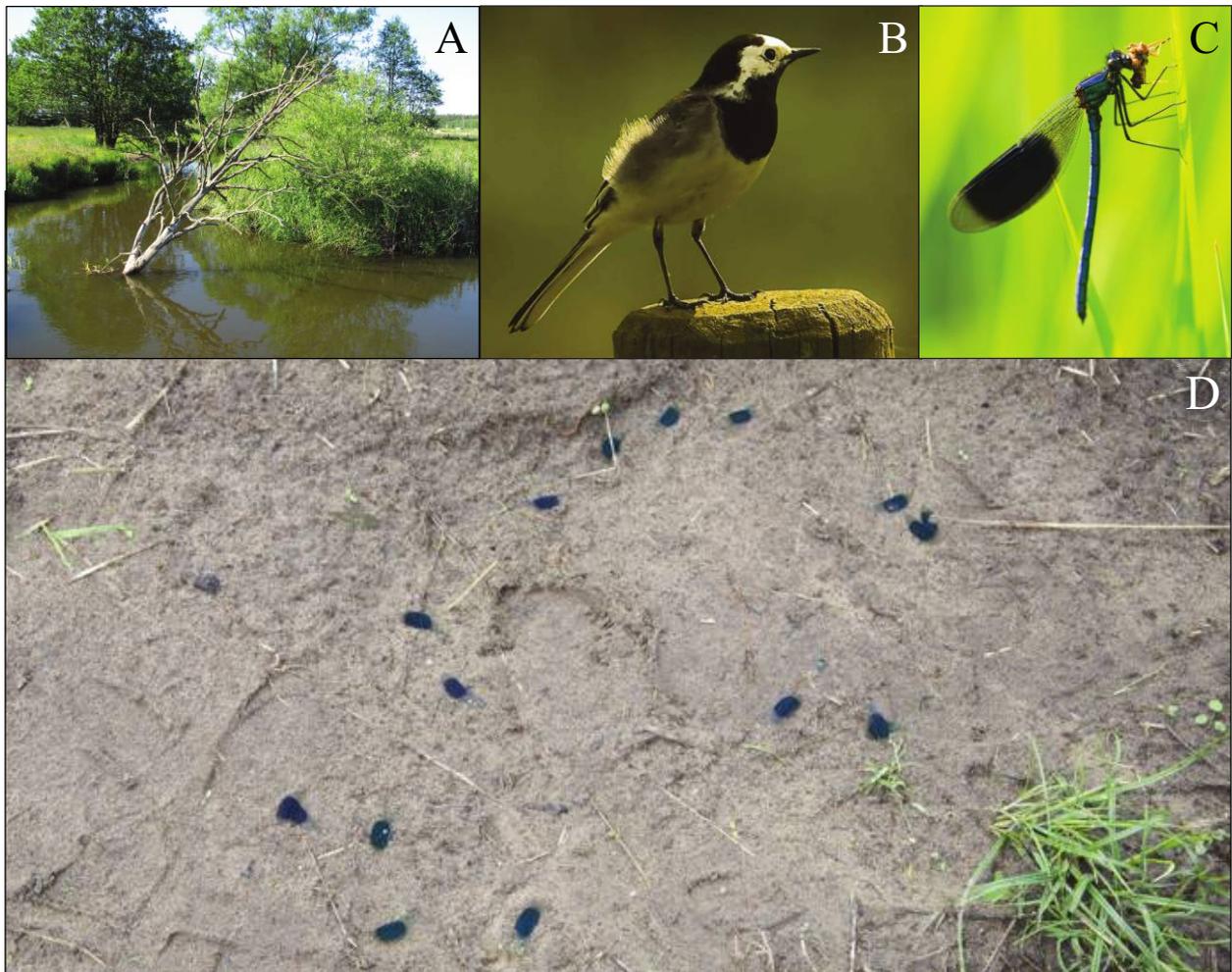


Figure 1: A, Bend in the river Klingavälsån, in Klingavälsån Naturreservat, Sweden. B, White wagtail. C, Male *Calopteryx splendens*. D, Slaughter station. This is a rather extreme example. In most instances, wings are few in number and more widely spaced out, allowing us to easily sort them according to individual. See text for more information.

(Haller and Hendry 2013). For instance, if trait variation is low relative to the breadth of a fitness peak, only directional selection may be detected. Thus, a finding of directional selection alone may not refute the existence of stabilizing selection. Moreover, directional and stabilizing selection do not necessarily exclude each other but can operate simultaneously, depending on the location of the population mean relative to the fitness peak (Lande and Arnold 1983). The continued study of traits expected to be under stabilizing selection is therefore needed, especially as stabilizing selection within populations may underlie patterns of divergence on macroevolutionary timescales and impact the long-term dynamics of the adaptive landscape (Estes and Arnold 2007; Uyeda et al. 2011; Hansen 2012; Svensson and Calsbeek 2012).

In contrast to traits that have been shaped by long-term predator-prey interactions, traits that perform other functions, such as sexually selected traits, may entail increased predation risk as a trade-off (Stuart-Fox et al. 2003; Hernandez-Jimenez and Rios-Cardenas 2012; but see McCullough et al. 2012). Antagonistic selection can thereby result in a population being displaced from its adaptive peak with regard to some components of fitness. This is the underlying theory behind evolutionary models involving fitness trade-offs. For example, Fisher's runaway model of sexual selection posits that sexually selected traits will exaggerate until counterbalanced by other forms of selection, such as predation (Fisher 1930). We therefore expect to find directional selection for less exaggerated trait values on sexually selected traits (i.e., less conspicuous wing patches) when the fitness component is predator-mediated viability. Consistent with this expectation, the evolutionary loss of elaborate male traits is more widespread than the gain of such traits, suggesting that direct selection against conspicuous signaling traits may be widespread (Wiens 2001; Svensson and Waller 2013). Finally, if traits related to flight performance are near their adaptive peaks with regard to predator-mediated viability selection, while traits under sexual selection are displaced from these adaptive peaks, selection should be stronger on the secondary sexual traits.

In addition to addressing the strength and mode of selection on wing traits, we also document differences in selection between the forewings and hindwings. This is of interest because damselflies (suborder Zygoptera), unlike dragonflies (suborder Anisoptera), have forewings and hindwings that are nearly identical in size and shape (Corbet 1999). Differences in selection between the fore- and hindwings thus raise the question of what evolutionary dynamics are involved in maintaining the similarity between the wings, including a consideration of flight performance and developmental constraints (Wake and Roth 1989; Arnold 1992). Here we report significant selection

for subtle differences in wing shape, which pertains to the issue of just how small a selectable domain of morphology can be. Studies of *Drosophila* wings have shown significant genetic variation for the evolution of very small morphological changes (Weber 1992), suggesting that a rapid evolutionary response to such selection may be expected given appropriate selective regimes and ecological conditions. Our study adds important ecological data needed to evaluate the potential for small, adaptive evolutionary changes in shape and contributes to the ongoing discussion about selection and the evolvability of insect wings (Hansen and Houle 2004, 2008).

Material and Methods

Study Species and the Natural History of Predation

Damselflies (Odonata: Zygoptera) in the genus *Calopteryx* (family Calopterygidae) are relatively large and showy damselflies with metallic bodies and broad, densely veined wings (Svensson and Waller 2013). Five *Calopteryx* species are found in Europe, two of which extend northward into Scandinavia, *C. splendens* and *C. virgo* (Dijkstra and Lewington 2006). They are found along streams and rivers and are often syntopic, including at the site of this study (Svensson and Friberg 2007). We focused on *C. splendens* in this study because it is more abundant and thus more amenable for estimating selection coefficients accurately (Brodie et al. 1995).

Our study population was located at Klingavälsån Naturreservat in southern Sweden (55.638°N, 13.542°E), along a 468-m stretch of the river that was surveyed daily (except when inclement weather precluded damselfly activity) from June 1, 2008, to July 17, 2008 (fig. 1). This is the same survey stretch used in previous studies of sexual selection and mate choice in *C. splendens* (Svensson et al. 2006, 2007; Svensson and Friberg 2007). We conducted a cross-sectional study (Lande and Arnold 1983) that compared the wings from randomly sampled live individuals to wings recovered at slaughter stations. Approximately 20–25 live *C. splendens* males were captured every day and their wings photographed. After photographing the wings, individuals were marked with unique color codes on the last three abdominal segments (see Svensson et al. 2004, 2006), which prevented their remeasurement on subsequent days. It is possible that wings recovered from slaughter stations belonged to live individuals measured earlier, but this would have been a rare event given the large population size of *C. splendens* at Klingavälsån Naturreservat. Wings from slaughter stations were collected daily and sorted to individual in the field by considering the exact collection locality within a slaughter station, wing and patch sizes, and wing patch color and opacity. In

almost every case this sorting was straightforward because daily sampling prevented the accumulation of large numbers of wings at each slaughter station. To avoid pseudoreplication, the sorting of wings at slaughter stations was done as conservatively as possible. For example, 12 wings were considered to be from no more than three individuals, unless there was compelling visual evidence that more than three individuals were present. Wings with unclear affinities were not analyzed.

White wagtails are the major predator of *C. splendens* at Klingavälsån (discussed in detail in Svensson and Friberg 2007). Wagtails are tetrachromatic, ultraviolet-sensitive birds that can easily see the blues and blacks present on *Calopteryx* damselflies (Bennett and Cuthill 1994; Avilés 2008). Only white wagtails have been observed depredating *Calopteryx* at Klingavälsåns Naturreservat, despite years of careful observation (Svensson et al. 2004, 2006; Svensson and Friberg 2007).

Wing Outline Shape and Size and Wing Patch Characteristics

A total of 967 wings were included in this study, including 335 forewings and 332 hindwings from live individuals, and 154 forewings and 146 hindwings from slaughter stations (all damaged wings were excluded). Live samples and samples from slaughter stations were combined for the entire field season (46 days). The size and shape of wings were quantified using geometric morphometric (GMM) techniques (Zelditch et al. 2012) as well as traditional biometric methods (wing length, wing width, and wing patch length). GMM methods, which use landmark-based data to capture the geometry of shape, are a multivariate approach to shape analysis that is generally superior to methods based on linear measurements, angles, or ratios (Adams et al. 2004). However, earlier work on *Calopteryx* wings employed length and width data collected using digital calipers (Svensson and Friberg 2007). By including traditional biometric data in this study, we were better able to compare our results with earlier work (the wings in earlier studies were not photographed, which precluded analysis of them using GMM techniques).

For GMM analyses, photos were taken using a Panasonic Lumix DMC-TZ3 camera. Wings collected from slaughter stations were photographed in the laboratory, while live individuals were photographed in the field and released. Wings from live individuals were photographed by pressing a forewing and hindwing simultaneously against a flat surface, and holding them flat with a transparency. Photos were taken with the help of a tripod set at a 90° angle relative to the wings. A scale bar was included in all photos. For the GMM analyses, 24 landmarks were placed on each wing (fig. 2) using the program tpsDig

(Rohlf 2008). All wings were processed by S. R. Kuchta only. Sixteen of the landmarks were chosen to capture the outline of the wing, and eight were chosen to quantify the size of the wing patch. The wings of *C. splendens* possess a dense network of veins, several of which run from the proximal end of the wing out to the wing margin. Consequently, there are a number of potential landmarks if one were to trace individual veins across the wing surface, however, in our photos it was often not possible to reliably track individual veins through the melanized wing patch. Thus, we chose outline methods for this study. Thirteen of the 16 wing landmarks were designated as semilandmarks. True landmarks represent discrete homologous points, whereas semilandmarks are placed on an outline according to extrinsic criteria and can slide during Procrustes superimposition (Bookstein 1997). Our three landmarks were located (1) at the junction of the R4+5, MA, and aculus veins (Askew 2004); (2) at the nodus on the anterior edge of the wing; and (3) at the apex of the wing (fig. 2A). Semilandmarks were placed with reference to standardized grids superimposed over the wing (described in detail in fig. A1; figs. A1–A4 available online). Because of the great care taken in creating a system for placing the semilandmarks precisely, the semilandmarks slid very little during the superimposition process.

The color patch was demarcated by placing eight landmarks by eye at standardized locations along the edge of the wing patch (landmark placement described in fig. 2B). The locations of these points are not homologous landmarks, nor are they good semilandmarks. We used these points only to quantify the size, not the shape, of the patch. Wing size and wing patch size were measured as the square root of the sum of squared distances between each individual landmark and the centroid of the shape.

Wing patch opacity was scored on a scale of 1 to 10 (by increments of 0.5) by holding a damselfly wing 1 cm above a black dot and comparing the visibility of the dot through the color patch to a set of black dots set against a gradient of progressively darker backgrounds. The color standards used to score patch opacity are provided in figure A2. All scoring was done outdoors under direct sunlight. Initially, all wings were scored independently by three people, but the method proved to be highly repeatable between observers and during the latter half of the field season most wings were scored by a single person (however, all three workers contributed equally). Wings that were difficult to score were scored by committee.

Prior to statistical analyses, a generalized Procrustes analysis (GPA) was used to eliminate variation due to size, position, and orientation. All remaining variation among configurations of landmarks is, by definition, shape variation (Kendall 1977). Shape variables were generated by projecting the GPA-corrected landmarks into a Euclidean

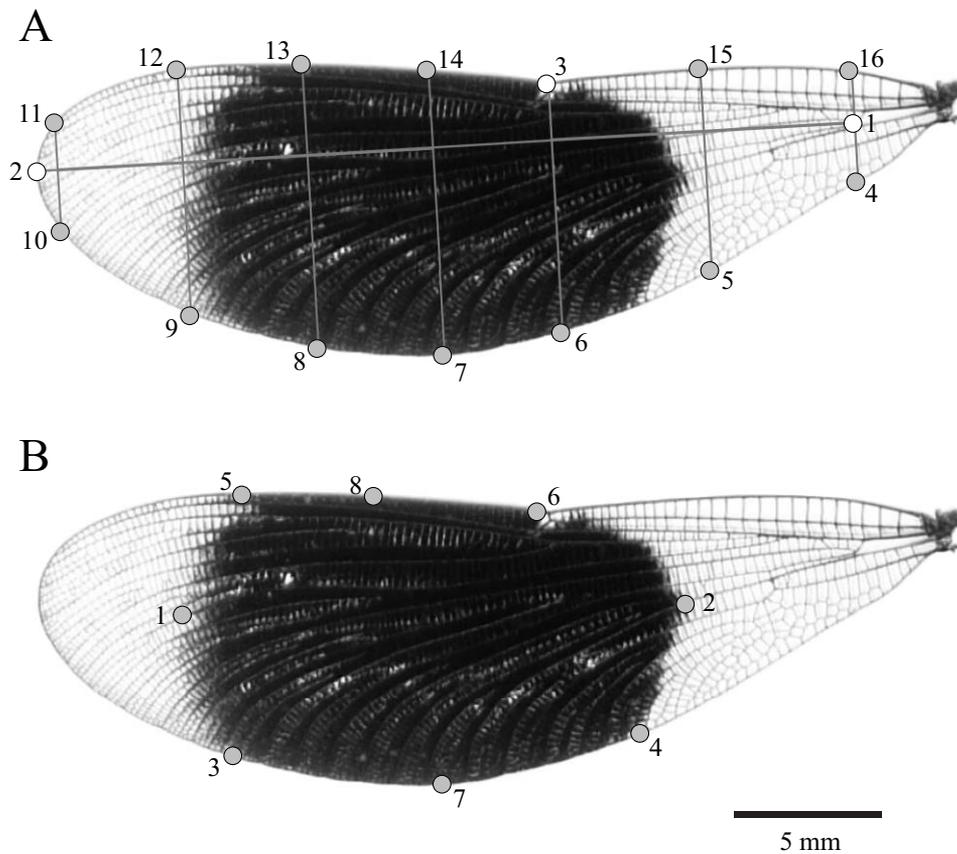


Figure 2: A, Forewing showing the placement of landmarks. White dots designate type I and type II landmarks (Bookstein 1991): 1 is located at the junction of the R4+5, MA, and acrilus veins (RMA junction); 2 is located at the apex of the wing; and 3 is located at the nodus. The gray dots capture the outline of the wing and are designated as semilandmarks. The placement of semilandmarks was facilitated by the placement of a series of grids and lines over the wing (see fig. A2, available online, for a detailed description of the grid placement techniques). B, Landmarks around the wing patch were placed by eye and were used only to calculate the centroid size of the patch. Landmark locations: along the proximal and distal edge of the wing patch in the center of the wing (landmarks 1, 2); where the proximal and distal edges of the wing patch meet the anterior and posterior sides of the wing (landmarks 3–6); and along the anterior and posterior wing margins, with landmark 7 corresponding to landmark 7 in A and landmark 8 midway between landmarks 13–14 in A.

shape space tangent to the Procrustes shape space (Dryden and Mardia 1998; Bookstein 1991) in tpsRelw (Rohlf 2007). Shape variables were subjected to a principal components analysis in tpsRelw, and all axes of variation that accounted for greater than zero percent of the variation were saved (15 axes). The centroid size of the wing outline and color patch were saved for later analyses.

Distinguishing Forewings and Hindwings

Whereas the forewings and hindwings of dragonflies (Anisoptera) exhibit large differences in shape and venation, they are nearly identical in damselflies (Zygoptera). This creates difficulties for categorizing wings found at slaughter stations. If forewings and hindwings can be distinguished, it is best to analyze them separately because

selection may operate on them differently. A discriminant function analysis on fore- and hindwings sampled from live individuals was used to determine whether forewings and hindwings can be distinguished reliably. Leave-out-one cross validation analysis (Lachenbruch 1967) conducted using the lda function in the MASS package in R v.2.15.3 (R Development Core Team 2013) resulted in six misclassified wings (99.1% classification success, out of 661 wings; fig. A3). This high success rate was not expected, as separating forewings and hindwings by eye is very difficult. Given the success at sorting wings from live individuals using GMM, wings from slaughter stations were categorized as either forewings or hindwings using a discriminant function in R. One forewing and one hindwing were randomly sampled from each individual at each slaughter station.

Covariation between Wing Traits and Survival

In this article, we refer to estimated selection gradients as either directional, stabilizing, disruptive, or correlational, and we refer to the coefficients in our statistical models as either linear or quadratic (but see Brodie et al. 1995). The relationship between wing shape variation and survival was assessed three ways. First, general linear models (GLMs) were used to calculate selection gradients for directional (β), stabilizing/disruptive (γ_{ii}), and correlational (γ_{ij}) forms of selection (Lande and Arnold 1983). For all analyses, forewings and hindwings were analyzed separately, with all independent variables (including principal component [PC] axes, wing size, and wing patch characteristics) standardized to have a mean of zero and unit variance (Lande and Arnold 1983). Mean-standardized selection gradients were not explored because they lack a natural interpretation when the data are not on a true ratio scale, which excludes PC axes (Hereford et al. 2004; Kingsolver and Pfennig 2007), and because the vast majority of selection studies have used variance-standardized selection gradients (Kingsolver et al. 2001; Gómez et al. 2006, 2008). Relative fitness for selection analyses was calculated as absolute fitness divided by mean fitness. In predation studies, individuals are either alive or dead, and the mean absolute fitness is thus equal to the proportion of individuals depredated, which must be estimated using independent observational information (Brodie 1992; Brodie and Janzen 1996; Janzen and Stern 1998). In this study, we performed daily surveys at the study site and recorded 4,495 live individuals and 230 depredated individuals, for a predation rate of 4.9% (this is similar to the rate of Svensson and Friberg 2007, who estimated a predation rate of 2.7% in 2002–2003). This may be a conservative estimate of the predation rate as it does not consider that live individuals may have been recounted on consecutive days (the average longevity of males at Klinavälsån is 4–5 days; Svensson et al. 2006). On the other hand, no daily survey could ever record every live animal at the study site, and thus the daily surveys underestimate the real population size. Overall, it is likely that the selection gradients reported in this study are minimum estimates, even though we report moderate to strong selection on many traits (cf. Hoekstra et al. 2001; Kingsolver et al. 2001).

Our estimated predation rate of 4.9% corresponds to a mean fitness of 0.95 and a relative fitness of 1.05 for living damselflies. To quantify selection coefficients, we assigned a relative fitness of 1.0 to live individuals and zero for those recovered at slaughter stations. Selection gradients (β) were estimated from the standardized partial regression coefficients of relative fitness (viability) on wing size, wing patch size, wing patch opacity, and the first three principal component axes of wing shape, which accounted for >80% of

the variation in phenotype (note that principal components are frequently referred to as relative warps in the GMM literature; Viscosi and Cardini 2011). Directional selection gradients (linear coefficients) were calculated from statistical models that included only linear terms, because if data are not multivariate normal linear and nonlinear coefficients will be correlated. Stabilizing/disruptive selection gradients and correlational selection gradients (nonlinear coefficients) were calculated from statistical models that included both linear and quadratic terms (Brodie 1992). Quadratic coefficients were doubled because GLMs underestimate stabilizing/disruptive selection gradients by half (Stinchcombe and Hoekstra 2008). Parameters were estimated using the `lm` function in R (R Development Core Team 2013), and confidence intervals were estimated using the `confint` function. Significance values for selection gradients were calculated using generalized linear models (GLZs) with a logit link function using the `glm` function in R. GLZs were used because while parametric statistics provide robust estimates of selection gradients and other parameters, the calculation of critical values may be violated by survival data, which tend to have nonnormally distributed errors (Mitchell-Olds and Shaw 1987; Brodie and Janzen 1996; Janzen and Stern 1998; Calsbeek and Kuchta 2011). Cubic splines illustrating the relationship between phenotypic characters and fitness were estimated in R using the `gam` function in the `mgcv` package. Each spline was fit separately to each trait. We calculated the splines and their standard errors using general additive models, including binomially distributed random errors and a logit link function (Schluter 1988), with 95% confidence intervals (CIs) calculated using Bayesian methods. To determine how closely the spline tracked the data, the smoothing parameter that minimized the generalized cross validation score was used. This maximizes the predictive value of the fitted model (Schluter 1988).

To determine whether levels of selection differed between performance related traits (wing shape and size) and sexually selected traits (wing patch size and opacity), we used a two-factor ANOVA with the absolute value of selection gradients used as the independent variable. Separate ANOVAs were used on direct and stabilizing/disruptive selection gradients.

To compare our results with those of a study by Svensson and Friberg (2007), we also conducted a complementary selection analysis using linear measures. Wing length was estimated as the distance between the landmark at the junction of the R4+5, MA, and acrolus veins and the apex of the wing (landmarks 1 and 2; fig. 2A). Wing width was estimated as the distance between the posterior-most landmark on the wing margin and the corresponding landmark on the anterior margin (landmarks 7 and 14; fig. 2A). Wing color patch length was estimated as the distance between

the landmarks centered at the proximal and distal edges of the wing patch (landmarks 7 and 8; figs. 2B, A1).

One concern regarding the use of GMM shape variation in traditional selection analyses is that shape is multidimensional (Bookstein 1991), and thus principal component axes are not separate variables with distinct biological meanings (Adams et al. 2004; Klingenberg and Monteiro 2005; Mitteroecker and Bookstein 2011). In addition, there is no guarantee that the major axes of shape variation will line up with the axes of shape variation along which selection is acting. On the other hand, it is common for researchers using traditional morphometrics to treat individual principal component axes as traits in selection analyses (e.g., Lande and Arnold 1983; Gómez et al. 2006, 2008; Gosden and Svensson 2008). In this article, we analyzed PC axes separately for comparative purposes, while acknowledging that the totality of shape is only obtained when all PC axes are analyzed simultaneously. To accommodate a multivariate perspective, we employed two-block partial least-squares (PLS) analysis to quantify the relationship between shape variation and fitness (Rohlf and Corti 2000; Gómez et al. 2006). PLS uses singular value decomposition to analyze the covariance structure underlying blocks of variables. Blocks can be considered latent variables (i.e., nonmeasured multidimensional variables defined by a set of manifest variables) with the PLS analysis identifying the maximum correlation among them (Rohlf and Corti 2000). The result is that the linear pairs of variables with maximal covariance are identified. The PLS analysis was carried out in MorphoJ v.1.05 (Klingenberg 2010), after importing the aligned landmarks and semilandmarks from tpsRelw (this preserves the alignment of coordinates from tpsRelw; Monteiro and Nogueira 2011). The RV coefficient (a multivariate generalization of the squared Pearson correlation coefficient, which takes a value between zero and one; Escoufier 1973) was used to quantify the association between fitness and shape. The null hypothesis of complete independence between the two blocks of variables (viability vs. shape) was tested using a permutation test with 10,000 replicates. The correlation coefficient (r) between the PLS scores for shape and fitness was also calculated. Forewings and hindwings were analyzed separately.

Data used in the selection analyses are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.sf804> (Kuchta and Svensson 2013).

Results

Shape Differences between Forewings and Hindwings

Wing shape was characterized with a set of 16 landmarks using geometric morphometric methods (fig. 2). A discriminant function analysis of the shape variables resulted

in 99.1% accuracy in correctly classifying forewings and hindwings (fig. A3). Thin-plate spline visualization revealed that the forewings were relatively long and narrow with a contracted region at the distal portion of the anterior end of the wing. There was also a more prominent notch or kink at the nodus. Hindwings, on the other hand, were relatively short and wide with an expanded region at the anterior end of the wing and a reduced notch at the nodus (fig. A3).

Principal components analysis of wing shape showed that a small number of axes were sufficient to capture most shape variation. For the forewings the first three PC axes accounted for 36.0%, 24.2%, and 20.5% of the variance, for a total of 80.7%. For the hindwings the first three PC axes accounted for 37.6%, 24.4%, and 20.8% of the variance, for a total of 82.8%. In both the forewing and the hindwing, no axis beyond axis 3 accounted for more than 6.8% of the variation (fig. A4). Thus, only axes 1–3 were included in the calculation of selection gradients.

Selection on Overall Shape

A two-block partial least-squares (PLS) analysis was used to quantify the relationship between overall wing shape (i.e., including all the shape variables) and predator-mediated fitness. For the forewing, we found a significant correlation between shape and fitness ($r = 0.30$, $P < .0001$; $RV = 0.049$, $P < .0001$). Warped outline drawings illustrate that male damselflies with forewings that were relatively long and narrow and with a relatively indented notch at the nodus were depredated less often (fig. 3A). A highly significant correlation between shape and fitness was also detected in the hindwing ($r = 0.26$, $P = .0015$; RV coefficient = $.019$, $P < .0001$), with males possessing an expanded antero-distal region exhibiting higher survivorship (fig. 3B).

The results of the PLS analysis largely mirror the observed differences between the forewing and the hindwing as revealed by the discriminant function analysis (fig. A3). In live individuals, the forewings were found to be relatively long and narrow with an indented notch (fig. A3), and the PLS analysis found selection for a similarly shaped wing (fig. 3A). In contrast, the hindwing was found to be relatively short and wide, with an expanded antero-distal region (fig. A3), and the PLS analyses also found selection for an expanded antero-dorsal region (fig. 3B).

Selection Analyses on Geometric Morphometric Variables

For the forewings, the results of selection analyses are shown in table 1 and figures 4–6. Positive directional selection was observed on the first principal component axis (PC1), with males possessing longer, narrower wings ex-

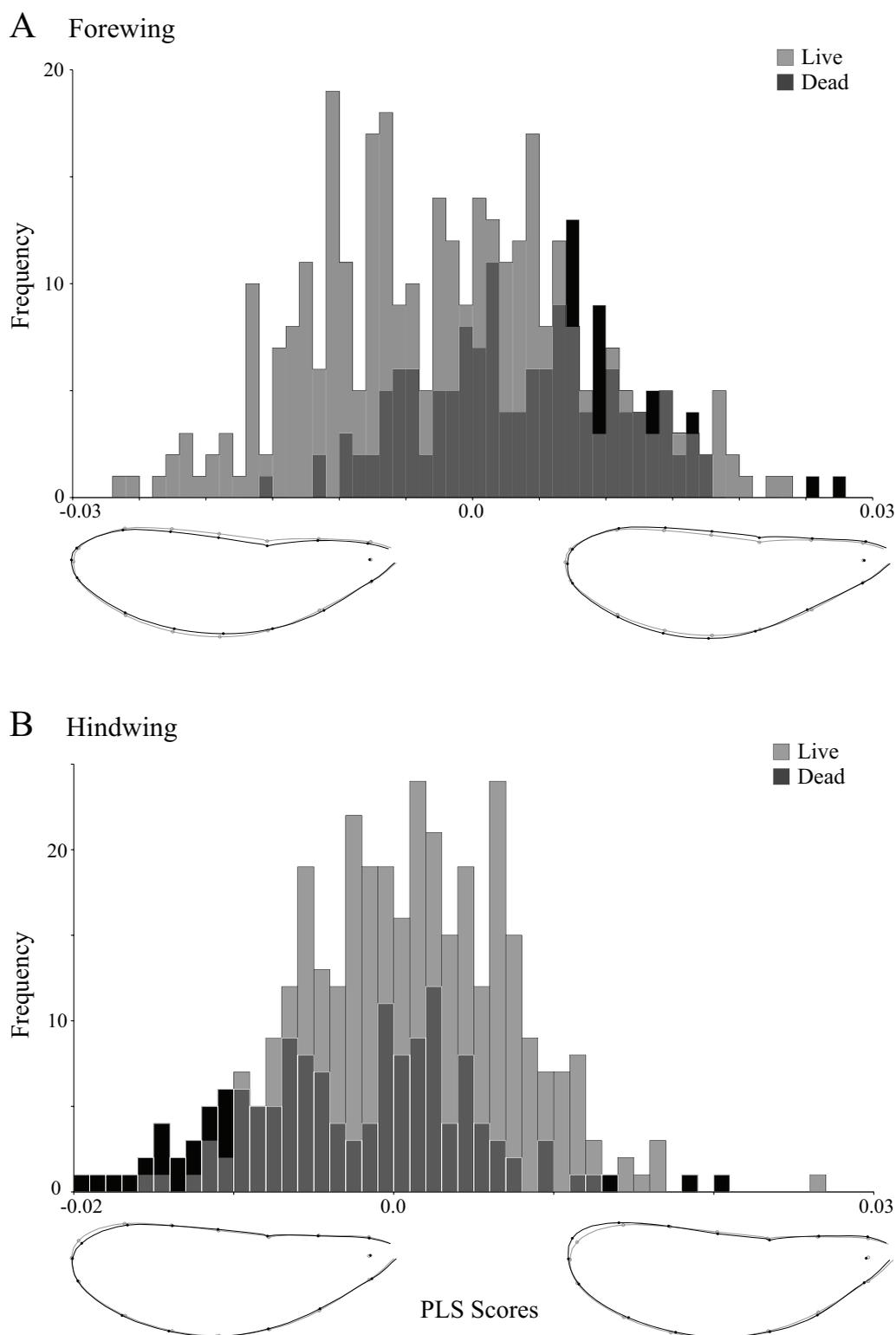


Figure 3: Results of the two-block partial least-squares analyses to estimate the selection differential (s) between shape and fitness. In the warped outline drawings, gray lines illustrate the average shape, and black lines illustrate extreme (but not exaggerated) shapes in the distribution. *A*, Forewings: selection on overall shape on forewings favored relatively long, narrow wings with a deep notch. *B*, Hindwings: selection on overall shape favored wings with an expanded anterior-distal region. PLS = partial least squares.

Table 1: Selection coefficients on the forewings of male *Calopteryx splendens*

	Selection differential <i>s</i>	Selection gradient β	PC1	PC2	PC3	Wing size	Patch opacity	Patch size
PC1	.098*** (.058, .138)	.090*** (.048, .132)	.049* (-.020, .119)					
PC2	.053* (.012, .094)	.029 (-.012, .070)	.048* (.005, .091)	<i>.004</i> (-.054, .063)				
PC3	.015 (-.027, .056)	.015 (-.025, .055)	.009 (-.033, .050)	-.003 (-.040, .033)	.069** (.010, .128)			
Wing size	.018 (-.024, .059)	-.045 [†] (-.091, .002)	.015 (-.036, .066)	-.006 (-.059, .047)	.051* (.006, .097)	-.177* (-.280, -.074)		
Patch opacity	-.105*** (-.147, -.063)	-.127*** (-.168, -.086)	.048 (.002, .094)	-.006 (-.048, .035)	.016 (-.027, .060)	.005 (-.046, .056)	-.136** (-.223, -.049)	
Patch size	.086*** (.045, .126)	.134*** (.087, .180)	-.029 (-.078, .021)	-.043 (-.094, .009)	-.054* (-.100, -.007)	.012 (-.044, .067)	.037 (-.014, .087)	-.094 (-.198, .011)

Note: On the left are columns of standardized directional selection differentials (*s*) and selection gradients (β). On the right is a matrix of standardized selection gradients, with stabilizing/disruptive selection (quadratic coefficients, γ_{ii}) in italics along the diagonal and correlational selection (γ_{ij}) below the diagonal. Quadratic coefficients have been multiplied by 2 to estimate stabilizing/disruptive selection gradients (Stinchcombe et al. 2008). In parentheses are 95% confidence intervals. PC = principal component axis. Values significant at $P < .05$ are indicated with asterisks and emphasized with bold.

[†] $P = .06$.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

hibiting higher survivorship than males with shorter, wider wings ($s = 0.098$ [95% CI: 0.058–0.138]; $\beta = 0.090$ [0.048–0.132]; fig. 3A). This is consistent with the PLS selection analysis, which revealed selection for long, narrow wings with an inset nodus (fig. 3A). Disruptive selection was also detected on PC1 of the forewing ($\gamma = 0.049$ [0.020–0.119]), though the dominant signal is directional (fig. 4A). For PC2, we found a positive selection differential ($s = 0.053$ [0.012–0.094]), but this was not observed using selection gradients, suggesting that selection along this axis of shape change is indirect (Lande and Arnold 1983). Selection on PC3 was disruptive ($\gamma = 0.069$ [0.010–0.128]; fig. 4B). Finally, selection on wing size was strongly stabilizing ($\gamma = -0.177$ [–0.280 to –0.074]), perhaps with a tendency toward favoring smaller wings on average ($\beta = -0.045$ [–0.091–0.002], $P = .06$; fig. 4C).

Selection on the size of the forewing color patch favored larger wing patches ($s = 0.086$ [0.045–0.126]; $\beta = 0.134$ [0.087–0.180]; fig. 5A), whereas selection on wing patch opacity favored more transparent wing patches ($s = -0.105$ [–0.147 to –0.063]; $\beta = -0.127$ [–0.168 to –0.086]; fig. 6A). In addition, we detected strong stabilizing selection on wing patch opacity in the forewing ($\gamma = -0.134$ [–0.223 to –0.049]; fig. 6A). Correlational selection was recorded between PC1 : PC2, PC3 : wing size, and PC3 : patch size, revealing that selection also operated on combinations of traits (table 1).

The results of selection analyses on the hindwing are shown in table 2 and figures 4–6. Neither PC1 nor PC2 were significantly correlated with viability, but PC3 experienced significant directional selection ($s = -0.067$

[–0.108 to –0.026]; $\beta = -0.053$ [–0.094 to –0.011]). A cubic spline analysis illustrates that wings expanded in the antero-dorsal dimension experienced reduced probabilities of predation (fig. 4D). This finding is consistent with the results from the PLS selection analysis, which also found selection favoring an expanded antero-distal region. Correlational selection acted on PC2 : PC3, PC1 : patch opacity, and PC2 : patch size, again showing that selection operated on combinations of traits (table 2). Finally, selection on wing size was not detected on the hindwing, in contrast with the forewing.

Directional selection on the size of the hindwing color patch favored larger patches ($s = 0.044$ [0.002–0.085]; $\beta = 0.060$ [0.012–0.108]) but overall was strongly stabilizing ($\gamma = -0.180$ [–0.287 to –0.072]; fig. 5B). In addition, selection on wing patch opacity favored individuals with more transparent wing patches ($s = -0.115$ [–0.157 to –0.073]; $\beta = -0.127$ [–0.169 to –0.085]), but here as well there was evidence of stabilizing selection overall ($\gamma = -0.173$ [–0.262 to –0.084]; fig. 6B).

In summary, selection on wing shape differed between forewings and hindwings, with selection favoring forewings that were relatively long and narrow and selection favoring hindwings with an antero-dorsal expansion. In contrast, natural selection on wing patch elements was more consistent, with selection favoring individuals with less opaque wing markings on both the fore- and hindwings. Stabilizing selection on patch opacity was detected in both wings as well. Lastly, individuals with larger wing patches in both their fore- and hindwings experienced

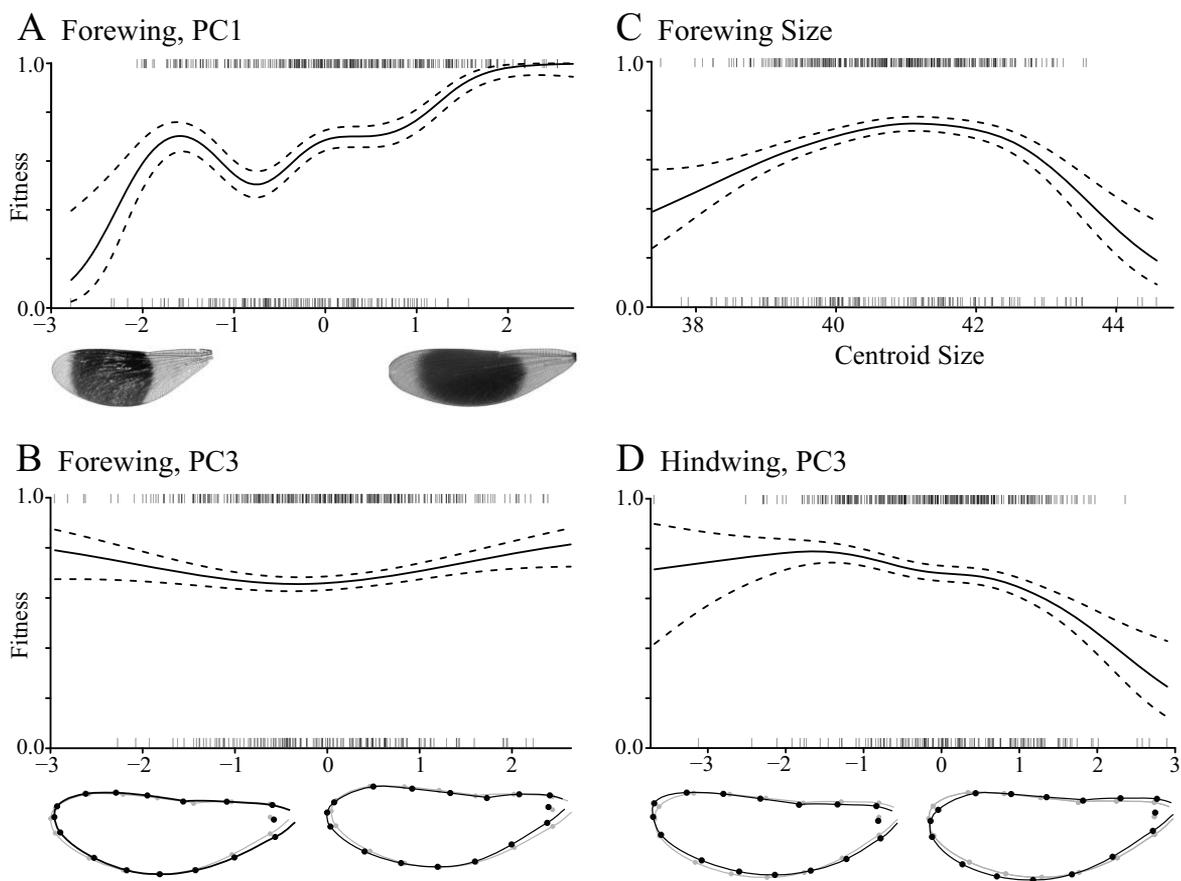


Figure 4: Cubic splines illustrating the relationship between morphological traits and fitness. The solid line is the spline, the dashed lines bound 95% confidence intervals, and the hashes at 0 and 1 represent dead and live individuals, respectively. Warped outline drawings were produced in MorphoJ (Klingenberg 2010), following a generalized Procrustes analysis in tpsRelw (Rohlf 2007). Extreme values are illustrated, but the shapes are not distorted beyond the range observed in the population. *A*, Forewing: relationship between PC1 and fitness. Representative wings are illustrated in place of warped outline drawings. *B*, Forewing: relationship between PC3 and fitness. *C*, Forewing: relationship between wing size and fitness. *D*, Hindwing: relationship between PC3 and fitness.

reduced risk of predation, but in the hindwing selection on wing patch size was overall stabilizing.

Selection on Shape Traits Versus Selection on Signaling Traits

We found that the strength and mode of selection on traits related to flight performance (wing size and shape) differed significantly from secondary sexual characters (wing patch size and opacity; tables 1, 2). Directional selection gradients on wing patch size and opacity were higher than directional selection gradients on wing shape and size (two-way ANOVA: performance traits vs. secondary sexual traits: $F = 18.771$, $df = 1$, $P = .003$; fore- vs. hindwing: $F = 2.468$, $df = 1$, $P =$ not significant [NS]; trait \times wing interaction: $F = 0.185$, $df = 1$, $P =$ NS; fig. 7). In addition, the strength of stabilizing/disruptive selection

was stronger on secondary sexual traits than it was on wing shape and size (two-way ANOVA: performance traits vs. secondary sexual traits: $F = 8.502$, $df = 1$, $P = .019$; fore- vs. hindwing: $F = 0.010$, $df = 1$, $P =$ NS; trait \times wing interaction: $F = 2.467$, $df = 1$, $P =$ NS; fig. 7). Descriptive statistics, including trait means, trait standard errors, and phenotypic variance-covariance and correlation matrices for the wing traits included in the selection data set are available in table A1, available online.

Selection Analyses on Traditional Morphometric Variables

For comparison with earlier studies, we combined the forewings and hindwings from our study and conducted selection analyses on linear measurements taken from these wings (wing length, wing width, and wing patch length; fig. 2; Svensson and Friberg 2007). The results are shown

Discussion

Wings are keystone traits in calopterygid damselflies (Svensson and Waller 2013). They function as the primary means of locomotion, influencing flight performance, foraging efficiency, and escape from aerial predators. In addition, color patches on the wings of many male calopterygids, including *Calopteryx splendens*, play an important role in male-male interactions, female choice, and species recognition (Siva-Jothy 1999; Svensson et al. 2004, 2006; Tynkkynen et al. 2004, 2005). Understanding how natural selection operates on secondary sexual traits is important for understanding trait diversification, as sexual selection is likely an important factor in speciation and the evolution of phenotypic novelty (West-Eberhard 1983; Corl et al. 2010b; Outomuro et al. 2012, 2013; Svensson and Waller 2013). Here we have investigated viability selection by wagtails on wing traits in male *C. splendens* with the aim of comparing the mode and strength of selection among traits.

Our selection analyses revealed that several traits experienced significant predator-mediated selection, in-

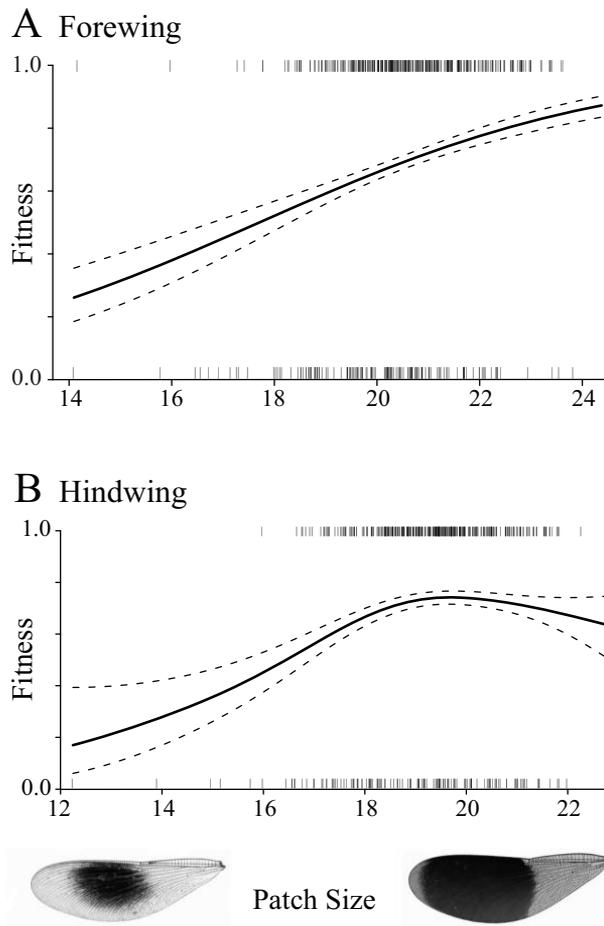


Figure 5: Cubic splines illustrating the relationship between wing patch size and fitness for the forewing (A) and hindwing (B). The solid line is the spline, the dashed lines bound 95% confidence intervals, and the hashes at 0 and 1 represent dead and live individuals, respectively. Representative wings from the population are shown.

in table 3. No significant directional selection on wing length and wing width was detected, however, strong stabilizing selection on these traits was revealed (wing length: $\gamma = -0.092$ [95% CI: -0.170 to -0.014]; wing width: $\gamma = -0.158$ [-0.224 to -0.092]). In addition, we found directional selection for larger wing patches ($s = 0.077$ [0.049–0.106]; $\beta = 0.123$ [0.090–0.157]) and less opaque wing patches ($s = -0.110$ [-0.139 to -0.080]; $\beta = -0.140$ [-0.169 to -0.110]). For both wing patch size and opacity, selection was overall strongly stabilizing (patch size: $\gamma = -0.134$ [-0.208 to -0.060]; patch opacity: $\gamma = -0.162$ [-0.223 to -0.100]). Finally, correlational selection was detected between wing length and patch opacity ($\gamma = 0.050$ [0.016–0.084]).

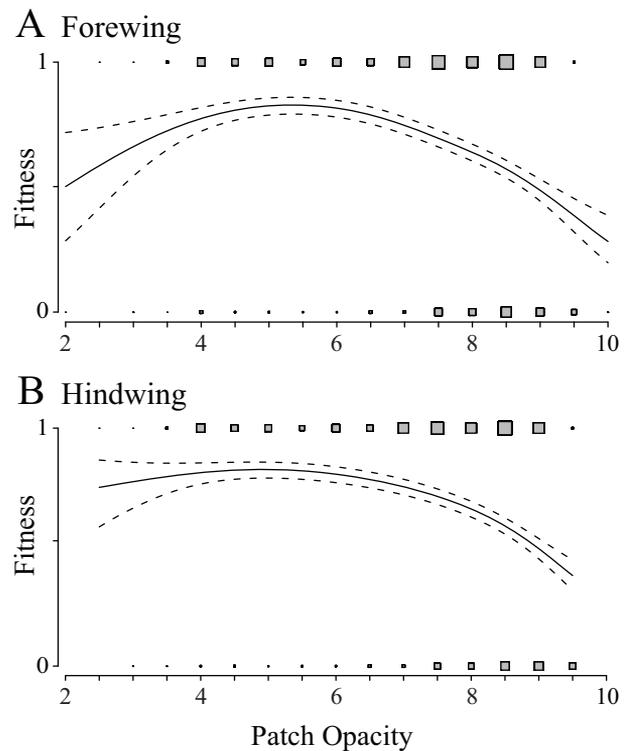


Figure 6: Cubic splines illustrating the relationship between wing patch opacity and fitness for the forewing (A) and hindwing (B). The solid line is the spline, and the dashed lines bound 95% confidence intervals. The boxes are sized proportional to the number of individuals sampled with each wing opacity score.

Table 2: Selection coefficients on the hindwings of male *Calopteryx splendens*

	Selection differential <i>s</i>	Selection gradient β	PC1	PC2	PC3	Wing size	Patch opacity	Patch size
PC1	.009 (-.032, .051)	-.005 (-.050, .041)	-.058 (-.126, .010)					
PC2	.03 (-.012, .071)	.028 (-.014, .069)	-.024 (-.066, .019)	.031 (-.028, .090)				
PC3	-.067** (-.108, -.026)	-.053* (-.094, -.011)	-.0004 (-.045, .045)	.059** (.016, .101)	-.038 (-.095, .019)			
Wing size	.018 (-.023, .060)	.008 (-.043, .059)	-.029 (-.086, .028)	.017 (-.030, .063)	.006 (-.047, .058)	-.062 (-.177, .053)		
Patch opacity	-.115*** (-.157, -.073)	-.127*** (-.169, -.085)	-.062* (-.110, -.014)	-.025 (-.068, .019)	-.017 (-.062, .028)	.005 (-.048, .059)	-.173*** (-.262, -.084)	
Patch size	.044* (.002, .085)	.060* (.012, .108)	-.029 (-.077, .020)	-.046* (-.090, -.002)	-.002 (-.051, .048)	.005 (-.057, .067)	.020 (-.031, .071)	-.180** (-.287, -.072)

Note: On the left are columns of standardized directional selection differentials (*s*) and selection gradients (β). On the right is a matrix of standardized selection gradients, with stabilizing/disruptive selection (quadratic coefficients, γ_{ii}) in italics along the diagonal and correlational selection (γ_{ij}) below the diagonal. Quadratic coefficients have been multiplied by 2 to estimate stabilizing/disruptive selection gradients (Stinchcombe et al. 2008). In parentheses are 95% confidence intervals. PC = principal component axis. Values significant at $P < .05$ are indicated with asterisks and emphasized with bold.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

cluding selection on wing size and shape (fig. 4; tables 1, 2) and selection on the opacity and size of the wing color patch (figs. 5, 6; tables 1, 2). Predator-mediated natural selection on traits related to flight performance (wing size and shape) was weaker than selection on traits related to sexual selection and species recognition (wing patch size and opacity; fig. 7). This difference in the strength of selection is consistent with our expectation that traits related to flight performance should be relatively optimized to reduce predation risk, whereas conspicuous secondary sexual traits will often entail a fitness trade-off with respect to predator-prey interactions (Svensson and Waller 2013).

While predator-mediated selection favored males with wing patches that were larger and less opaque than average, selection on patch opacity in the forewing and on patch size and opacity in the hindwing was overall strongly stabilizing (figs. 5, 6; tables 1, 2). The finding of widespread stabilizing selection on secondary sexual traits runs counter to our expectation that secondary sexual traits should be relatively displaced from their adaptive peak with respect to predator-prey interactions and thus primarily under strong directional selection. In some instances, secondary sexual traits may have positive effects on viability as well as mate choice and hence may be favored by both natural and sexual selection (Bonduriansky 2011), which could lead to stabilizing selection on an optimal trait value. For example, it has been proposed that dark wing patches in calopterygid damselflies could also be advantageous in terms of thermoregulation, although empirical evidence for this remains somewhat mixed (Svensson and Waller 2013). An alternative ex-

planation for the finding of stabilizing selection is that predation risk is an indirect effect of social interactions, with individuals possessing extreme wing patch traits being forced into situations that expose them to predators. For instance, it has been shown that successful territory holders possess higher levels of wing pigmentation (Grether 1996; Cordoba-Aguilar 2002). If males with prime territories are at more risk of predation (Toivanen et al. 2009), predator-mediated selection would select against males with large patches. Conversely, males with small, dim wing patches might be less efficient at avoiding predation attempts because such individuals are physiologically weak, possessing lower immunocompetence and higher fluctuating asymmetry (Rantala et al. 2000, 2011). Interspecific interactions could also play a role, as males of *C. splendens* with larger wing patches tend to be displaced from territories by the congeneric competitor *Calopteryx virgo* (Tynkkyinen et al. 2004, 2005, 2006). Thus, there are multiple mechanisms by which individuals with extreme secondary sexual traits might experience higher levels of predation, resulting in an overall pattern of stabilizing selection.

Comparison of Selection Analyses

While geometric morphometric methods are highly effective at quantifying shape and illustrating shape change (Adams et al. 2004; Klingenberg 2010), the use of GMM in studies of selection is made difficult by the fact that shape is multidimensional, yet individual PC axes are not independent biological characters with specific biological meanings (Klingenberg and Monteiro 2005; Mitteroecker

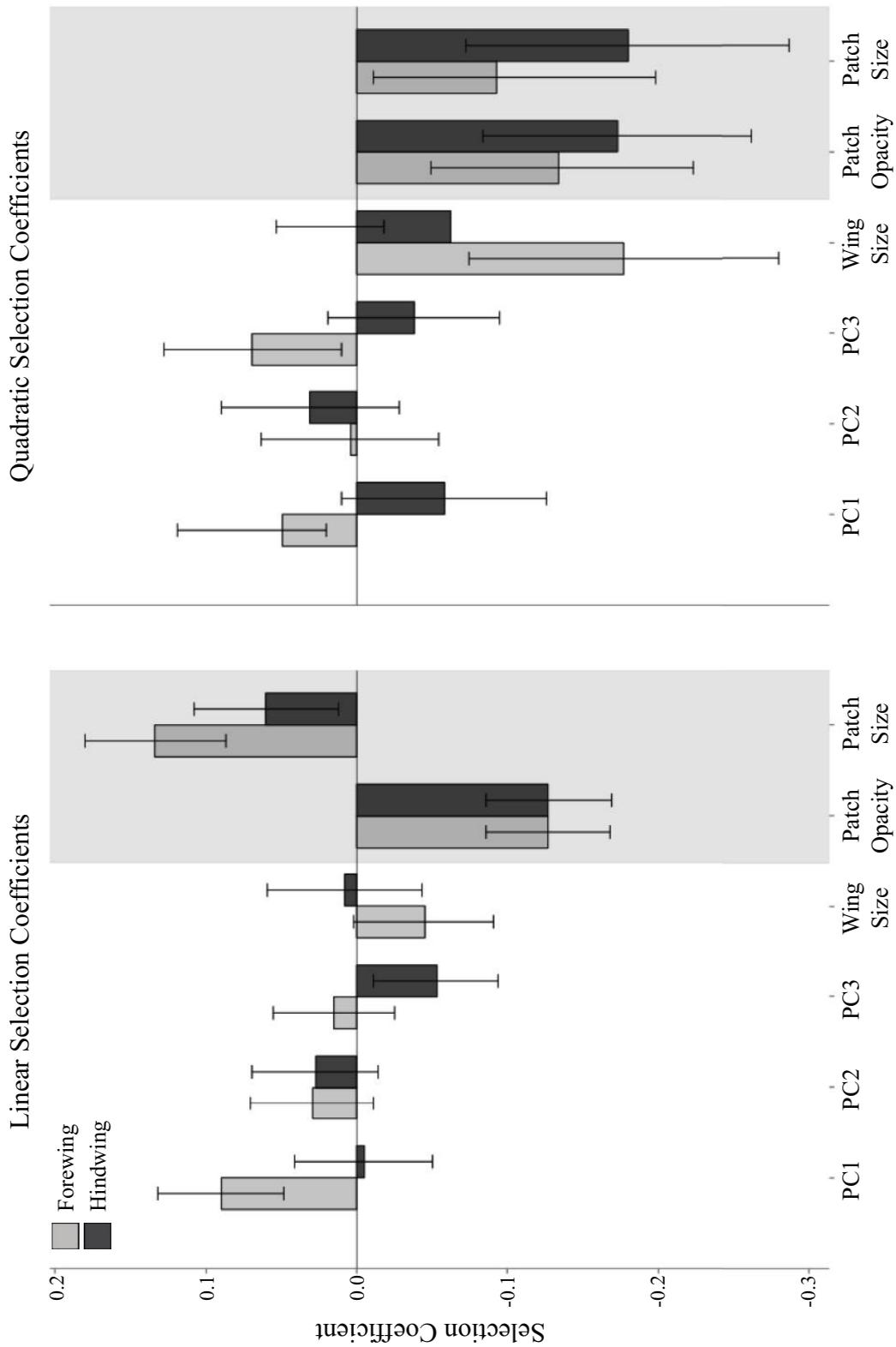


Figure 7: Bar chart summarizing the selection analyses, illustrating that selection gradients on wing patch characteristics are, on average, larger than are selection gradients on wing shape (PC1–PC3) and size. This is true for both linear and quadratic terms.

Table 3: Selection coefficients on linear measurements of male *Calopteryx splendens*, front and hindwings combined (see text)

	Selection differential s	Selection gradient β	Wing length	Wing width	Patch length	Patch opacity
Wing length	.018 (-.011, .047)	-.013 (-.048, .022)	<i>-.092*</i> (<i>-.170, -.014</i>)			
Wing width	-.011 (-.040, .018)	-.016 (-.047, .015)	.029 (-.008, .066)	<i>-.158***</i> (<i>-.224, -.092</i>)		
Patch length	.077*** (.049, .106)	.123*** (.090, .157)	.011 (-.029, .051)	-.021 (-.055, .013)	<i>-.134**</i> (<i>-.208, -.060</i>)	
Patch opacity	<i>-.110***</i> (<i>-.139, -.080</i>)	<i>-.140***</i> (<i>-.169, -.110</i>)	.050** (.016, .084)	-.038 [†] (-.070, -.005)	.022 (-.013, .057)	<i>-.162***</i> (<i>-.223, -.100</i>)

Note: On the left are columns of standardized directional selection differentials (s) and selection gradients (β). On the right is a matrix of standardized selection gradients, with stabilizing/disruptive selection (quadratic coefficients, γ_{ii}) in italics along the diagonal and correlational selection (γ_{ij}) below the diagonal. Quadratic coefficients have been multiplied by 2 to estimate stabilizing/disruptive selection gradients (Stinchcombe et al. 2008). In parentheses are 95% confidence intervals. Values significant at $P < .05$ are indicated with asterisks and emphasized with bold.

[†] $P = .06$.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

and Bookstein 2011; Zelditch et al. 2012). We advocate caution when interpreting individual PC axes in traditional selection analyses. In our analyses, we conducted selection analyses using individual PC axes primarily to document selection on elements of shape, not to predict the response to selection or calculate a rate of change. A potential benefit of estimating directional selection on PC axes is that these axes reflect linear combinations of traits and thus include elements of correlational selection. GMM approaches might thus possess good statistical power for detecting correlational selection, in contrast with traditional selection analyses of linear traits, which require significant two-way interactions to document correlational selection (Brodie 1992; Sinervo and Svensson 2002). An additional advantage of traditional selection analyses (using linear measures or PC axes) over available multidimensional approaches (such as PLS) is that traditional analyses can estimate stabilizing/disruptive selection and correlational selection, and they can estimate selection gradients instead of selection differentials (Lande and Arnold 1983). Finally, traditional selection analyses readily incorporate other independent elements of the phenotype, such as wing patch opacity and size.

To account for multivariate selection on wings, we used a two-block partial least-squares (PLS) analysis to estimate the selection differential (s) between shape and fitness (fig. 3). PLS analyses estimate the covariance between two sets of variables, in this case all of the principal components versus viability. The results were strikingly similar to our traditional selection analyses in that they revealed selection for a forewing that is relatively long and narrow with a more strongly indented nodus, and for a hindwing with

an antero-dorsal expansion near the apex of the wing (fig. 3).

Finally, in this study we found that even minor size and shape differences are associated with significant differences in viability (figs. 3–6; tables 1–3). Some of these shape differences would be hard to detect by the eye, such as the subtle differences in shape between the fore- and hindwings (fig. A3). Our study of selection on wing traits applies to the question of what is the smallest selectable domain, a problem raised by Weber (1992) in his analysis of the genetics of wing shape in *Drosophila melanogaster* (see also Hansen and Houle 2004). Weber (1992) showed that there is significant genetic variation along almost all wing shape dimensions, suggesting that the wing has the potential to evolve in almost any direction, including evolutionary changes in very small (<0.2 mm) and adjacent (<0.3 mm) regions of the wing surface. That natural selection by a predator in the wild can “see” subtle differences in shape, as we captured by our GMM analyses (tables 1, 2), has not been much studied. Our results suggest that predator-mediated selection can indeed operate on subtle differences in wing shape, and if the results of Weber (1992) are general, these differences in selection would result in adaptive differentiation if not checked by other factors.

Forewings, Hindwings, and Flight Biomechanics

The traditional selection analyses and the PLS analyses both indicate that natural selection favored individuals with fore- and hindwing shapes that are exaggerations of the differences in shape that already exist between the

wings (figs. 3, 4, A3). Among odonates, damselflies have very similar fore- and hindwings, whereas dragonflies have differently shaped fore- and hindwings. These differences impact flight performance, though the relationship between shape and performance is complex (Marden 2008; Wootton and Newman 2008). In general, damselflies have higher stroke amplitudes, lower wing beat frequencies, and lower accelerations and velocities than do dragonflies (Rüppell 1989). For instance, Wakeling and Ellington (1997) compared the flight performance of *C. splendens* with the dragonfly *Sympetrum sanguineum*, which has a similar body mass and wing area. They found that the two species traveled at similar velocities, however, the wingbeat frequency of *C. splendens* was half that of *S. sanguineum*, while *C. splendens* accelerated more per wingbeat and flew further per wingbeat. The main distinction is that *C. splendens* (and damselflies in general) generated large amounts of lift using a “clap and fling” mechanism, where the wings on either side of the body come together broadly at the dorsal-most position of the upstroke, and then fling apart on the downstroke (Rudolph 1976). Using this mechanism, damselflies create greater mass-specific lift than do dragonflies (Marden 1987). Dragonflies, which do not use the clap and fling mechanism, tend to fly with their wings beating completely out of phase: the wings do not touch at the top of the stroke, and the forewings lag behind the hindwings by $\sim 90^\circ$. Some research suggests that the differentiation between the wings in dragonflies has improved their flight speed and maneuverability (Wootton and Newman 2008), but the biomechanics of flight in odonates remains an active field of study and the interactions between the forewing and hindwing during flight are not well understood (Wakeling and Ellington 1997). Our results indicate there is selection for increased differentiation between the wing pairs in *C. splendens*. If our findings are general, this suggests that selection for differentiation in wing shape may be offset by other factors, such as performance trade-offs or developmental constraints (Wake and Roth 1989; Arnold 1992).

Fluctuating Selection despite a Common Selective Agent

There are notable differences between this study and a previous study on *C. splendens* at the same locality (Svensson and Friberg 2007). In the earlier study, linear measurements (wing length and width, wing patch length, and wing patch darkness) were used to quantify selection. Forewings and hindwings were combined in the analyses, and the sampling spanned two field seasons (2002–2003). This earlier study found evidence for directional selection favoring shorter, wider wings, as well as correlational selection creating a fitness ridge connecting short, narrow wings

to long, wide wings. This differs from the results of our study, in which selection favored long, narrow wings over short, wide wings in the forewing, while in the hindwing selection favored wings with an expansion at the anterio-distal region (fig. 4; table 2).

To what extent are these differences in selection related to differences in methodology, as opposed to real biological differences between the years? To answer this question, we combined our data on forewings and hindwings and analyzed selection on wing length, wing width, wing patch length, and patch opacity (table 3). In this new data set, we did not detect directional selection on wing length or wing width, however, we did find significant stabilizing selection on both of these traits. This latter result is broadly consistent with Svensson and Friberg (2007), who found stabilizing selection corresponding to a fitness ridge. However, this analysis also showed that combining forewings and hindwings obscured differences in selection between the wings. In this study, separating forewings and hindwings and using GMM led to novel insights regarding the nature of selection on wing shape and revealed subtle differences in selection that were not detected when wings were combined.

This study also differs from Svensson and Friberg (2007) with respect to the mode of selection on secondary sexual traits. While both studies found that selection favored individuals with less opaque wing patches, Svensson and Friberg (2007) showed that selection on wing patch opacity was disruptive, favoring the lightest and darkest wing patches, whereas in our study, selection on patch opacity was stabilizing for both the forewings and the hindwings (fig. 6). This is also true for analyses based on linear measures alone (table 3). In addition, while Svensson and Friberg (2007) did not find significant selection on wing patch length, this study documented selection favoring larger wing patches on both the fore- and hindwings, with stabilizing selection in the hindwing patch. Selection for a longer wing patch was also found using linear measures (table 3). In sum, despite the fact that the agent of selection is known (predation by wagtails), selection seems to fluctuate temporally in this system.

Thousands of estimates of selection have been published over the last 3 decades, following the seminal paper by Lande and Arnold (1983). This has been summarized in several influential meta-analyses, which have greatly improve our understanding of the strength and mode of selection in natural populations (Hoekstra et al. 2001; Kingsolver et al. 2001; Siepielski et al. 2009, 2011, 2013; Kingsolver and Diamond 2011). These meta-analyses indicate that selection is pervasive and strong enough to result in rapid evolutionary change, given the presence of additive genetic variation for the traits under selection. However, a recent review of studies that quantified mul-

multiple episodes of selection found that selection commonly fluctuates across years, including changes of both magnitude and direction (Siepielski et al. 2009). Morrissey and Hadfield (2012) argue that Siepielski et al. (2009) overestimated the tendency for selective pressures to flip-flop over time; nonetheless, there is little doubt that selection often varies across space and time (Grant and Grant 2002; Thompson 2005; Gosden and Svensson 2008; Feldman et al. 2009), with profound evolutionary consequences (Calsbeek et al. 2012). Biotic factors that lead to variability in selection include frequency-dependent and density-dependent selection, sexual conflict, chase-away selection, and coevolutionary arms races between predators and prey (Thompson 2005; Gosden and Svensson 2008; Feldman et al. 2009; Calsbeek et al. 2012). This dynamic view of selection differs from earlier, more static views of the adaptive landscape, where selection was implicitly assumed to be constant over evolutionary time (Dietrich and Skipper 2012; Svensson and Calsbeek 2012). Understanding the conditions under which selection is stable versus when it is fluctuating is fundamental to our understanding of anagenetic change, adaptation, and speciation (Estes and Arnold 2007; Uyeda et al. 2011; Calsbeek et al. 2012; Haller and Hendry 2013). In our studies of *C. splendens*, we have found that predator-mediated selection on wing shape was relatively stable across two study periods, whereas predator-mediated selection on secondary sexual characters was more labile. To better understand the causes of fluctuating selection on traits we need a more detailed understanding of the ecology of selection (Wade and Kalisz 1990; MacColl 2011), including knowledge about selective agents such as predators (Young et al. 2004; Cunningham et al. 2013), abiotic factors such as extreme weather events (Grant and Grant 2002), and interactions with conspecifics (Corl et al. 2010a).

One strength of this study is that the agent of selection (predation by wagtails) has been clearly identified, which is not the case for the majority of selection studies (MacColl 2011). Given that we know the agent of selection, we might have expected selection to be relatively consistent between years, compared to studies in which the agents of selection are unknown (but see Thompson 2005). However, both our current and past research on predator-mediated selection in *C. splendens* indicates that this is not necessarily the case. Even when the agent of selection is known, the relationship between phenotype and fitness is still often dynamic and not easily predicted (Grant and Grant 2002).

Acknowledgments

Financial support was provided by Ohio University and the National Science Foundation (DEB-0601971; to

S.R.K.) and by grants from the Swedish Research Council (to V.R.) and Gyllenstiernska Krapperupsstiftelsen, Kungliga Vetenskapsakademien (KVA), and Carl Tryggers Stiftelse (CTS; to E. I. S.). We are grateful to the Kuchta-Roosenburg-White lab meeting participants at Ohio University. We also thank T. Bourdier and K. Moeller, who labored as cheerful field assistants, and D. Adams, F. Eroukhmanoff, T. Gosden, and M. Wellenreuther for numerous discussions during the design and implementation of this project. A. Runemark provided critical and constructive suggestions on the first draft of this manuscript.

Literature Cited

- Adams, D. C., F. J. Rohlf, and D. Slice. 2004. Geometric morphometrics: ten years of progress following the "revolution." *Italian Journal of Zoology* 71:5–16.
- Allen, B. J., and J. S. Levinton. 2007. Costs of bearing a sexually selected ornamental weapon in a fiddler crab. *Functional Ecology* 21:154–161.
- Arnold, S. J. 1992. Constraints on phenotypic evolution. *American Naturalist* 140(suppl.):S85–S107.
- Askew, R. R. 2004. *The dragonflies of Europe*. Revised ed. Harley, Colchester.
- Avilés, J. M. 2008. Egg colour mimicry in the common cuckoo *Cuculus canorus* as revealed by modelling host retinal function. *Proceedings of the Royal Society B: Biological Sciences* 275:2345–2352.
- Bennett, A. T., and I. C. Cuthill. 1994. Ultraviolet vision in birds: what is its function? *Vision Research* 34:1471–1478.
- Bonduriansky, R. 2011. Sexual selection and conflict as engines of ecological diversification. *American Naturalist* 178:729–745.
- Bookstein, F. 1991. *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press, Cambridge.
- . 1997. Landmark methods for forms without landmarks: localizing group differences in outline shape. *Medical Image Analysis* 3:279–289.
- Brodie, E. D., III. 1992. Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. *Evolution* 46:1284–1298.
- Brodie, E. D., III, and F. J. Janzen. 1996. On the assignment of fitness values in statistical analyses of selection. *Evolution* 50:437–442.
- Brodie, E. D., III, A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. *Trends in Ecology and Evolution* 10:313–318.
- Brown, C. R., and M. B. Brown. 2013. Where has all the road kill gone? *Current Biology* 23:R233–R234.
- Calsbeek, R., and S. Kuchta. 2011. Predator mediated selection and the impact of developmental stage on viability in wood frog tadpoles (*Rana sylvatica*). *BMC Evolutionary Biology* 11:353.
- Calsbeek, R., T. Gosden, S. R. Kuchta, and E. I. Svensson. 2012. Fluctuating selection and dynamic adaptive landscapes. In E. I. Svensson and R. Calsbeek, eds. *The adaptive landscape in evolutionary biology*. Oxford University Press, Oxford.
- Clapham, M. E., and J. A. Karr. 2012. Environmental and biotic controls on the evolutionary history of insect body size. *Proceedings of the National Academy of Sciences of the USA* 109:10927–10930.

- Corbet, P. S. 1999. Dragonflies: behavior and ecology of Odonata. Comstock, Cornell University Press, Ithaca, NY.
- Cordoba-Aguilar, A. 2002. Wing pigmentation in territorial male damselflies, *Calopteryx haemorrhoidalis*: a possible relation to sexual selection. *Animal Behavior* 63:759–766.
- Corl, A., A. R. Davis, S. R. Kuchta, T. Comendant, and B. Sinervo. 2010a. Alternative mating strategies and the evolution of sexual size dimorphism in the side-blotched lizard, *Uta stansburiana*: a population-level comparative analysis. *Evolution* 64:79–96.
- Corl, A., A. R. Davis, S. R. Kuchta, and B. Sinervo. 2010b. Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proceedings of the National Academy of Sciences of the USA* 107:4254–4259.
- Cott, H. B. 1940. Adaptive coloration in animals. Oxford University Press, Oxford.
- Cunningham, C. J., G. T. Ruggerone, and T. P. Quinn. 2013. Size selectivity of predation by brown bears depends on the density of their sockeye salmon prey. *American Naturalist* 181:663–673.
- Dietrich, M. R., and R. A. Skipper Jr. 2012. A shifting terrain: a brief history of the adaptive landscape. Pages 1–15 in E. I. Svensson and R. Calsbeek, eds. *The adaptive landscape in evolutionary biology*. Oxford University Press, Oxford.
- Dijkstra, K.-D. B., and R. Lewington. 2006. Field guide to the dragonflies of Britain and Europe: including western Turkey and north-western Africa. *British Wildlife*, Gillingham.
- Dryden, I., and K. Mardia. 1998. *Statistical shape analysis*. Wiley, Chichester.
- Eroukhanoff, F., and E. I. Svensson. 2009. Contemporary parallel diversification, antipredator adaptations and phenotypic integration in an aquatic isopod. *PLoS ONE* 4:e6173.
- Escoufier, Y. 1973. Le traitement des variables vectorielles. *Biometrics* 29:751–760.
- Estes, S., and S. J. Arnold. 2007. Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. *American Naturalist* 169:227–244.
- Fairbairn, D. J., and J. P. Reeve. 2001. Natural selection. Pages 9–43 in C. W. Fox, D. A. Roff, and D. J. Fairbairn, eds. *Evolutionary ecology: concepts and case studies*. Oxford University Press, Oxford.
- Feldman, C. R., E. D. Brodie Jr., E. D. Brodie III, and M. E. Pfrender. 2009. The evolutionary origins of beneficial alleles during the repeated adaptation of garter snakes to deadly prey. *Proceedings of the National Academy of Sciences of the USA* 106:13415–13420.
- Fisher, R. A. 1930. *The genetical theory of natural selection*. Clarendon, Oxford.
- Godin, J.-G. J., and H. E. McDonough. 2003. Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behavioral Ecology* 14:194–200.
- Gómez, J., F. Perfectti, and J. P. M. Camacho. 2006. Natural selection on *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. *American Naturalist* 168:531–545.
- Gómez, J. M., J. Bosch, F. Perfectti, J. D. Fernández, M. Abdelaziz, and J. P. M. Camacho. 2008. Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. *Proceedings of the Royal Society B: Biological Sciences* 275:2241–2249.
- Gosden, T., and E. I. Svensson. 2008. Spatial and temporal dynamics in a sexual selection mosaic. *Evolution* 62:845–856.
- Grant, P. R., and B. R. Grant. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296:707–711.
- Grether, G. F. 1996. Intrasexual competition alone favors a sexually dimorphic ornament in the rubyspot damselfly *Hetaerina americana*. *Evolution* 50:1949–1957.
- Haller, B. C., and A. P. Hendry. 2013. Solving the paradox of stasis: squashed stabilizing selection and the limits of detection. *Evolution* 68:483–500.
- Hansen, T. F. 2012. Adaptive landscape and macroevolutionary dynamics. In E. I. Svensson and R. Calsbeek, eds. *The adaptive landscape in evolutionary biology*. Oxford University Press, Oxford.
- Hansen, T. F., and D. Houle. 2004. Evolvability, stabilizing selection, and the problem of stasis. Pages 130–150 in Pigliucci, M., and K. Preston, eds. *Phenotypic integration: studying the ecology and evolution of complex phenotypes*. Oxford University Press, Oxford.
- . 2008. Measuring and comparing evolvability and constraint in multivariate characters. *Journal of Evolutionary Biology* 21:1201–1219.
- Hereford, J., T. F. Hansen, and D. Houle. 2004. Comparing strengths of directional selection: how strong is strong? *Evolution* 58:2133–2143.
- Hernandez-Jimenez, A., and O. Rios-Cardenas. 2012. Natural versus sexual selection: predation risk in relation to body size and sexual ornaments in the green swordtail. *Animal Behavior* 84:2051–2059.
- Kingsolver, J. G., and D. W. Pfennig. 2007. Patterns and power of phenotypic selection in nature. *BioScience* 57:561–572.
- Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Beerli, and J. G. Kingsolver. 2001. Strength and tempo of directional selection in the wild. *Proceedings of the National Academy of Sciences of the USA* 98:9157–9160.
- Janzen, F. J., and H. S. Stern. 1998. Logistic regression for empirical studies of multivariate selection. *Evolution* 52:1564–1571.
- Kendall, D. 1977. The diffusion of shape. *Advances in Applied Probability* 9:428–430.
- Kingsolver, J. G., and S. E. Diamond. 2011. Phenotypic selection in natural populations: what limits directional selection? *American Naturalist* 177:346–357.
- Kingsolver, J. G., S. E. Diamond, A. M. Siepielski, and S. M. Carlson. 2012. Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. *Evolutionary Ecology* 26:1101–1118.
- Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. *American Naturalist* 157:245–261.
- Kingsolver, J. G., and D. W. Pfennig. 2007. Patterns and power of phenotypic selection in nature. *BioScience* 57:561–572.
- Klingenberg, C. P. 2010. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* 11:353–357.
- Klingenberg, C. P., and L. Monteiro. 2005. Distances and directions in multidimensional shape spaces: implications for morphometric applications. *Systematic Biology* 54:678–688.
- Kotiaho, J. S., R. V. Alatalo, J. Mappes, S. Parri, and A. Rivero. 1998. Male mating success and risk of predation in a wolf spider: a balance between sexual and natural selection? *Journal of Animal Ecology* 67:287–291.
- Kuchta, S. R. 2005. Experimental support for aposematic coloration in the salamander *Ensatina eschscholtzii xanthoptica*: implications for mimicry of Pacific newts. *Copeia* 2005:265–271.
- Kuchta, S. R., A. H. Krakauer, and B. Sinervo. 2008. Why does the

- yellow-eyed *Ensatina* have yellow eyes? Batesian mimicry of Pacific newts (genus *Taricha*) by the salamander *Ensatina eschscholtzii xanthoptica*. *Evolution* 62:984–990.
- Kuchta, S. R., and E. I. Svensson. 2014. Data from: Predator-mediated natural selection on the wings of the damselfly *Calopteryx splendens*: differences in selection among trait types. *American Naturalist*, Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.sf804>.
- Lachenbruch, P. 1967. An almost unbiased method of obtaining confidence intervals for probability of misclassification in discriminant analysis. *Biometrics* 23:639–645.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- MacColl, A. D. 2011. The ecological causes of evolution. *Trends in Ecology and Evolution* 26:514–522.
- Marden, J. H. 1987. Maximum lift production during takeoff in flying animals. *Journal of Experimental Biology* 130:235–258.
- . 2008. Dragonfly flight performance: a model system for biomechanics, physiological genetics, and animal competitive behaviour. Pages 249–259 in A. Córdoba-Aguilar, ed. *Dragonflies and damselflies: model organisms for ecological and evolutionary research*. Oxford University Press, Oxford.
- McCullough, E. L., P. R. Weingarden, and D. J. Emlen. 2012. Costs of elaborate weapons in a rhinoceros beetle: how difficult is it to fly with a big horn? *Behavioral Ecology* 23:1042–1048.
- Michael, P. H., J. Deutsch, and J. Burney. 1994. Size, dominance and copulatory success in male northern elephant seals, *Mirounga angustirostris*. *Animal Behavior* 48:1249–1260.
- Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* 41:1149–1161.
- Mitteroecker, P., and F. Bookstein. 2011. Linear discrimination, ordination, and the visualization of selection gradients in modern morphometrics. *Evolutionary Biology* 38:100–114.
- Monteiro, L. R., and M. R. Nogueira. 2011. Evolutionary patterns and processes in the radiation of phyllostomid bats. *BMC Evolutionary Biology* 11:137.
- Morrissey, M. B., and J. D. Hadfield. 2012. Directional selection in temporally replicated studies is remarkably consistent. *Evolution* 66:435–442.
- Otomuro, D., F. Bokma, and F. Johansson. 2012. Hind wing shape evolves faster than front wing shape in *Calopteryx* damselflies. *Evolutionary Biology* 39:116–125.
- Otomuro, D., D. C. Adams, and F. Johansson. 2013. The evolution of wing shape in ornamented-winged damselflies (Calopterygidae, Odonata). *Evolutionary Biology* 40:300–309.
- Panhuis, T. M., R. Butlin, M. Zuk, and T. Tregenza. 2001. Sexual selection and speciation. *Trends in Ecology and Evolution* 16:364–371.
- Plaistow, S., and M. T. Siva-Jothy. 1996. Energetic constraints and male mate-securing tactics in the damselfly *Calopteryx splendens xanthostoma* (Charpentier). *Proceedings of the Royal Society B: Biological Sciences* 263:1233–1239.
- R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org/>.
- Rantala, M. J., J. Koskimäki, J. Taskinen, K. Tynkkynen, and J. Suhonen. 2000. Immunocompetence, developmental stability and wingspot size in the damselfly *Calopteryx splendens* L. *Proceedings of the Royal Society B: Biological Sciences* 267:2453–2457.
- Rantala, M. J., J. Honkavaara, and J. Suhonen. 2010. Immune system activation interacts with territory-holding potential and increases predation of the damselfly *Calopteryx splendens* by birds. *Oecologia* (Berlin) 163:825–832.
- Rantala, M. J., J. Honkavaara, D. W. Dunn, and J. Suhonen. 2011. Predation selects for increased immune function in male damselflies, *Calopteryx splendens*. *Proceedings of the Royal Society B: Biological Sciences* 278:1231–1238.
- Reznick, D. N., F. H. Shaw, F. H. Rodd, and R. G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* 275:1934–1937.
- Ritchie, M. G. 2007. Sexual selection and speciation. *Annual Review of Ecology, Evolution, and Systematics* 38:79–102.
- Rohlf, F. 2007. tpsRelw, relative warps analysis, version 1.45. <http://life.bio.sunysb.edu/morph/>.
- . 2008. tpsDig2, digitize landmarks and outlines, version 2.12. <http://life.bio.sunysb.edu/morph/>.
- Rohlf, F., and M. Corti. 2000. Use of two-block partial least-squares to study covariation in shape. *Systematic Biology* 49:740–753.
- Rudolph, R. 1976. Some aspects of wing kinematics in *Calopteryx splendens* (Harris) (Zygoptera: Calopterygidae). *Odonatologica* 5: 119–127.
- Rüppell, G. 1989. Kinematic analysis of symmetrical flight manoeuvres of Odonata. *Journal of Experimental Biology* 144:13–42.
- Ryan, M. J., M. D. Tuttle, and A. S. Rand. 1982. Bat predation and sexual advertisement in a Neotropical anuran. *American Naturalist* 119:136–139.
- Simmons, R. E., and L. Scheepers. 1996. Winning by a neck: sexual selection in the evolution of giraffe. *American Naturalist* 148:771–786.
- Schillaci, M. A. 2006. Sexual selection and the evolution of brain size in primates. *PLoS ONE* 1:e62.
- Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* 42:849–861.
- Siepielski, A. M., J. D. Dibattista, and S. M. Carlson. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecology Letters* 12:1261–1276.
- Siepielski, A. M., J. D. Dibattista, J. A. Evans, and S. M. Carlson. 2011. Differences in the temporal dynamics of phenotypic selection among fitness components in the wild. *Proceedings of the Royal Society B: Biological Sciences* 278:1572–1580.
- Siepielski, A. M., K. M. Gotanda, M. B. Morrissey, S. E. Diamond, J. D. Dibattista, and S. M. Carlson. 2013. The spatial patterns of directional phenotypic selection. *Ecology Letters* 16:1382–1392.
- Sinervo, B., and E. I. Svensson. 2002. Correlational selection and the evolution of genomic architecture. *Heredity* 89:329–338.
- Siva-Jothy, M. T. 1999. Male wing pigmentation may affect reproductive success via female choice in a calopterygid damselfly (Zygoptera). *Behaviour* 136:10–11.
- . 2000. A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. *Proceedings of the Royal Society B: Biological Sciences* 267:2523–2527.
- Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? *Evolution* 62: 2435–2440.
- Stuart-Fox, D. M., A. Moussalli, N. J. Marshall, and I. P. Owens. 2003. Conspicuous males suffer higher predation risk: visual modelling and experimental evidence from lizards. *Animal Behavior* 66:541–550.

- Svensson, E. I., and R. Calsbeek. 2012. The adaptive landscape in evolutionary biology. Oxford University Press, Oxford.
- Svensson, E. I., F. Eroukhmanoff, and M. Friberg. 2006. Effects of natural and sexual selection on adaptive population divergence and premating isolation in a damselfly. *Evolution* 60:1242–1253.
- Svensson, E. I., and M. Friberg. 2007. Selective predation on wing morphology in sympatric damselflies. *American Naturalist* 170:101–112.
- Svensson, E. I., K. Karlsson, M. Friberg, and F. Eroukhmanoff. 2007. Gender differences in species recognition and the evolution of asymmetric sexual isolation. *Current Biology* 17:1943–1947.
- Svensson, E. I., L. Kristoffersen, K. Oskarsson, and S. Bensch. 2004. Molecular population divergence and sexual selection on morphology in the banded demoiselle (*Calopteryx splendens*). *Heredity* 93:423–433.
- Svensson, E. I., A. G. McAdam, and B. Sinervo. 2009. Intralocus sexual conflict over immune defense, gender load, and sex-specific signaling in a natural lizard population. *Evolution* 63:3124–3135.
- Svensson, E. I., and J. T. Waller. 2013. Ecology and sexual selection: evolution of wing pigmentation in calopterygid damselflies in relation to latitude, sexual dimorphism and speciation. *American Naturalist* 182:E174–E195.
- Thompson, J. N. 2005. The geographic mosaic theory of coevolution. University of Chicago Press, Chicago.
- Toivanen, T., M. J. Rantala, and J. Suhonen. 2009. Influence of alternative mating tactics on predation risk in the damselfly *Calopteryx virgo*. *Canadian Journal of Zoology* 87:684–688.
- Tynkkynen, K., J. S. Kotiaho, M. Luojumäki, and J. Suhonen. 2005. Interspecific aggression causes negative selection on sexual characters. *Evolution* 59:1838–1843.
- . 2006. Interspecific territoriality in *Calopteryx* damselflies: the role of secondary sexual characters. *Animal Behavior* 71:299–306.
- Tynkkynen, K., M. J. Rantala, and J. Suhonen. 2004. Interspecific aggression and character displacement in the damselfly *Calopteryx splendens*. *Journal of Evolutionary Biology* 17:759–767.
- Uyeda, J. C., T. F. Hansen, S. J. Arnold, and J. Pienaar. 2011. The million-year wait for macroevolutionary bursts. *Proceedings of the National Academy of Sciences of the USA* 108:15908–15913.
- Viscosi, V., and A. Cardini. 2011. Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. *PLoS ONE* 6:e25630.
- Wade, M. J., and S. Kalisz. 1990. The causes of natural selection. *Evolution* 44:1947–1955.
- Wake, D. B., and G. Roth. 1989. Complex organismal functions: integration and evolution in vertebrates. Wiley, New York.
- Wakeling, J. M., and C. P. Ellington. 1997. Dragonfly flight. II. Velocities, accelerations and kinematics of flapping flight. *Journal of Experimental Biology* 200:557–582.
- Weber, K. E. 1992. How small are the smallest selectable domains of form? *Genetics* 130:345–353.
- West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. *Quarterly Review of Biology* 58:155–183.
- Wheeler, B. 2010. lmpPerm: permutation tests for linear models. R package version 1.1-2. <http://CRAN.R-project.org/package=lmpPerm>
- Wiens, J. J. 2001. Widespread loss of sexually selected traits: how the peacock lost its spots. *Trends in Ecology and Evolution* 16:517–523.
- Wootton, R. J., and D. J. Newman. 2008. Evolution, diversification, and mechanics of dragonfly wings. Pages 261–274 in A. Cordoba-Aguilar, ed. *Dragonflies and damselflies: model organisms for ecological and evolutionary research*. Oxford University Press, Oxford.
- Young, K. V., E. D. Brodie Jr., and E. D. Brodie III. 2004. How the horned lizard got its horns. *Science* 304:65–65.
- Zelditch, M., D. Swiderski, and H. D. Sheets. 2012. Geometric morphometrics for biologists: a primer. 2nd ed. Academic Press, San Diego, CA.
- Zuk, M., and G. R. Kolluru. 1998. Exploitation of sexual signals by predators and parasitoids. *Quarterly Review of Biology* 73:415–438.

Associate Editor: David N. Reznick
Editor: Troy Day