

Directional and fluctuating asymmetry in the black-winged damselfly *Calopteryx maculata* (Beauvois) (Odonata: Calopterygidae)

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Abstract: Directional asymmetry (DA) has received considerably less attention than fluctuating asymmetry (FA) in the literature. Evidence for DA, however, is building among insect taxa. We examined asymmetries in two wing traits within both sexes of the damselfly *Calopteryx maculata* (Beauvois) (Odonata: Calopterygidae) sampled from three sites in southeastern Ontario. After accounting for measurement error, we show that proximal segments within right fore and hind wings are consistently longer than those in the left in all but one sample group. Full wing lengths, however, exhibited FA rather than DA. Mean asymmetry values for both traits (segment and length) occurred in the direction of right-wingedness significantly more often than expected by chance. Patterns of asymmetry were generally consistent among the sexes and sites, although males tended to exhibit more pronounced DA. We suggest that the wings of *C. maculata* may undergo compensatory development, so that full lengths are more bilaterally symmetrical than their component parts.

Résumé : L'asymétrie directionnelle (DA) est un phénomène beaucoup moins étudié que l'asymétrie fluctuante (FA) dans la littérature. Cependant, nous avons de plus en plus de preuves que l'asymétrie directionnelle prévaut chez les insectes. Nous avons étudié l'asymétrie de deux caractéristiques des ailes chez des mâles et des femelles de la Demoiselle bistrée, *Calopteryx maculata* (Beauvois) (Odonata : Calopterygidae) échantillonnés à trois endroits dans le sud-est de l'Ontario. En tenant compte des erreurs de mesures, nous avons constaté que les segments proximaux des ailes antérieure et postérieure droites étaient toujours plus longs que dans les ailes de gauche, chez tous les groupes échantillonnés sauf un. La longueur totale des ailes, toutefois, subit une asymétrie fluctuante plutôt que directionnelle. Les valeurs moyennes de l'asymétrie dans les deux cas (segments et longueur totale) tendent à favoriser l'aile droite significativement plus souvent que si elles étaient dues au hasard. L'asymétrie est généralement semblable chez les deux sexes et à tous les sites, mais les mâles font preuve d'une asymétrie directionnelle plus importante. Il est possible que les ailes de *C. maculata* subissent un développement de compensation qui fait que la longueur totale a une symétrie bilatérale plus importante que les composantes des ailes.

[Traduit par la Rédaction]

Introduction

Of the possible forms of subtle asymmetry in bilateral characters, fluctuating asymmetry (FA) has received the most attention (e.g., Palmer 1994; Leung and Forbes 1996; Møller and Swaddle 1997). FA is defined as subtle random deviations from perfect symmetry (Van Valen 1962). FA arises when the stabilising processes inherent in organism development (i.e., developmental homeostasis) are unable to buffer against disruptive factors during development (Palmer 1996). Increased levels of FA, beyond those which are expected solely from the interplay of developmental "noise" and stabilising processes, have been correlated with extreme conditions of environment (e.g., high levels of pollutants; Graham et al. 1993) and restricted gene flow (e.g., inbreeding or genetic bottlenecks; Leary et al. 1985; Wayne et al. 1986). Its potential as a tool for monitoring stress levels in natural pop-

ulations, and as an indicator of individual quality or fitness (e.g., Leung and Forbes 1996; Møller 1997), has thus prompted this attention (Leary and Allendorf 1989; Clarke 1993; Graham et al. 1993; Palmer 1996). Subtle directional asymmetry (DA), the case when one side's character value is consistently greater than the other, has received considerably less attention (e.g., Palmer 1994; Kraak 1997; Møller and Swaddle 1997; Rowe et al. 1997). We are aware of only a handful of studies that document the existence of significant DA within insects (crickets, Simmons and Ritchie 1996; honeybees, Smith et al. 1997; houseflies, Goulson et al. 1999; fruit flies, house flies, and tsetse flies, Klingenberg et al. 1998; wood butterfly; Windig and Nylin 1999; bumblebees; C.P. Klingenberg, personal communication). The degree to which methodological problems (Palmer and Strobeck 1997) have served to bias the literature away from reporting DA is unknown but possibly significant (Rowe et al. 1997). This bias may have been enhanced by the fact that DA is commonly deemed uninterpretable in relation to stress or fitness relations (the most common goal of FA studies; Graham et al. 1998). Indeed, whether DA can, in fact, be used in any way to infer developmental stability is still debated (e.g., Palmer 1994; Møller and Thornhill 1997; Palmer and Strobeck 1997; Graham et al. 1998).

Consider, for example, the case of FA in damselflies (Odonata: Zygoptera). Prior to 1997, several studies purported

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to find ideal FA in wing length and reported that FA was correlated with measures of individual fitness and (or) quality (Harvey and Walsh 1993; Córdoba-Aguilar 1995; Bonn et al. 1996). However, none of these studies properly evaluated the contributions of both measurement error and other forms of asymmetry (i.e., DA) to their quantification of FA. Interestingly, subsequent experiments that used appropriate methodology found inconsistent relationships between FA and measures of individual fitness and quality (Forbes et al. 1997; Leung and Forbes 1997) and between FA and environmental stress (Hardersen and Wratten 1998; Hardersen et al. 1999). Furthermore, using these more appropriate methods, Leung and Forbes (1997) and Hardersen et al. (1999) found some evidence for DA in the wings of their subject species. Thus, whether or not differences in damselfly wing lengths conform to a distribution consistent with ideal FA (normal or leptokurtic distribution with mean zero; Palmer and Strobeck 1992) is debatable. Indeed, given the recent findings of DA in several insect taxa (above), it could be that shortcomings in methodology were masking a phenomenon that is widespread in the insect world (Klingenberg et al. 1998).

As part of a previous study relating wing morphology to landscape structure (Pither 1997), we found preliminary evidence suggesting the presence of DA in the wings of the damselfly *Calopteryx maculata* (Beauvois) (Odonata: Calopterygidae) within several populations sampled in Nova Scotia. Here we present the results of a follow-up study, conducted in eastern Ontario, that set out to more explicitly examine patterns of asymmetry in the wings of males and females of this species. We determine whether FA or DA is present within multiple traits, and compare patterns of asymmetry among the sexes and three sample sites.

Materials and methods

Sample collections

Twenty-five male and 25 female *C. maculata* were collected from each of three streams in the vicinity of the Queen's University Biological Station (QUBS) in eastern Ontario, Canada: Forsyth Creek (henceforth called Forsyth; 391 000 m E, 4 933 300 m N UTM co-ordinates), Peterson Creek (Peterson; 3 942 000 m E, 4 933 000 m N), and Black Creek (Black; 390 200 m E, 4 954 500 m N). Only reproductively mature individuals were collected (adult and immature damselflies are easily distinguished visually by eye), and all sampling was conducted over a short period of time within 14 days of the first emergence recorded at each of the three sites. The maturation period for this species is typically 7–11 days (Waage 1972). Collection dates (all in 1998) were 28 May (Peterson), 31 May (Black), and between 29 May and 1 June (Forsyth). A standard insect flight net was used, and individuals were placed within mesh cages upon capture. Within 2 h of capture all individuals were placed in vials and stored in a freezer at -10°C until measured. All individuals received identical treatment throughout.

Measurements

The fore wings and hind wings of each individual were clipped at their base and fastened between two acetate sheets (forming an envelope) using clear adhesive tape. The first millimetre of the proximal end of each wing was taped to one of the sheets. Each envelope of acetate sheets (containing 15–18 individuals' wings) was scanned once at a resolution of 600 dpi using a Hewlett Packard 4c Scanjet flatbed scanner with a background light source (i.e., a transparency adapter). Scanning at this resolution produces images

with pixel dimensions of 0.05×0.05 mm. All measurements are presented in millimetres.

The scanned images of the wings were enlarged on the computer screen to approximately 11 times their normal size and were measured using an on-screen pointer that is part of a freeware image-analysis software program (Image Tools; University of Texas Health Sciences Center 1997). On all individuals, we measured a segment of the wing that runs parallel to the R_1 vein of the fore and hind wings, henceforth referred to as the "segment" (Fig. 1). On a subset of the individuals, we measured a trait that better represents the full length of the wing, henceforth referred to as the "length" (Fig. 1). The pigmentation of the distal portions of male wings was too dark to measure wing length on all individuals (some wings were also slightly damaged distally). Thus, for 14 males and 14 females (to maintain balanced sample sizes) from each site (a total of 42 individuals/sex), we performed this second measurement on the fore and hind wings. All measurements were taken twice (using the same image, see below) by the same person, each time in a haphazard order.

Measurement error

Two potential sources of measurement error exist: human measurement error and error due to the scanning process. The former source of error was evaluated for both traits using mixed-model ANOVAs, following the methods of Palmer and Strobeck (1986). To test for both potential sources of error simultaneously (thus providing an additional test of the human measurement error), we scanned a total of 18 individual wings (nine left and nine right of a pair) twice, at orientations perpendicular to each other, and repeated segment measurements (in a haphazard order) for each scanned image twice. We then ran a mixed-model ANOVA with "scan" and "individual" each as a random effect (individual nested within scan) and repeated measurements as the residual error. This enabled a partitioning of variance components due to the scanning effect (random effect 1, 1 df), the difference in segment size among individuals (random effect 2, 16 df), and within-individual measurement errors (residual error, 18 df) (cf. Yezerinac et al. 1992). This entire exercise was conducted for each sex. We also scanned five individual female wings from the front and back (i.e., flip sides), and conducted a similar procedure. We additionally tested for a difference in calculated left- minus right-wing values of the segments (average of two left-wing measurements minus average of two right-wing measurements) for the two "flip-side" scans.

Testing for normality

Asymmetry for each trait was taken as the average of left-wing minus the average of right-wing repeated measurements. The resulting asymmetry data for each site and sex were tested for normality using a combination of skewness (G_1) and kurtosis (G_2) statistics (Zar 1999) and graphical techniques.

Patterns of asymmetry among sites and sexes

For each trait (fore-wing and hind-wing segment and length asymmetry, total of four traits) and group (males and females within each of three sites), we conducted mixed-model ANOVAs and t tests, to evaluate the significance of different forms of asymmetries and departures from a mean of zero asymmetry, respectively. Beyond establishing if a particular group's mean asymmetry value was different from zero, we were interested in whether the direction of asymmetry was consistent among the different sample groups. To do this, we used a binomial test, wherein the null hypothesis was that positive and negative values of mean group asymmetries were equally likely, and should thus occur at a ratio of 1:1. We calculated the magnitude of trait asymmetry as a percentage of mean trait size (a scale-independent measure that permits comparisons with other studies) by dividing a group's mean absolute value for left minus right by its mean character length (Klingenberg et al.

Fig. 1. Landmarks used for measuring wing segments (short white line) and lengths (long white line), shown here on a left fore wing from a female *Calopteryx maculata*. Labels follow nomenclature in Walker (1953): ar, arculus; R₁, radial vein 1; M₁, median vein branch 1; and sn, subnodus. The segments were measured from the intersection of M₁ and the subnodal vein to the arculus, and the lengths were measured from the distal terminus of the M₁ vein to the arculus.

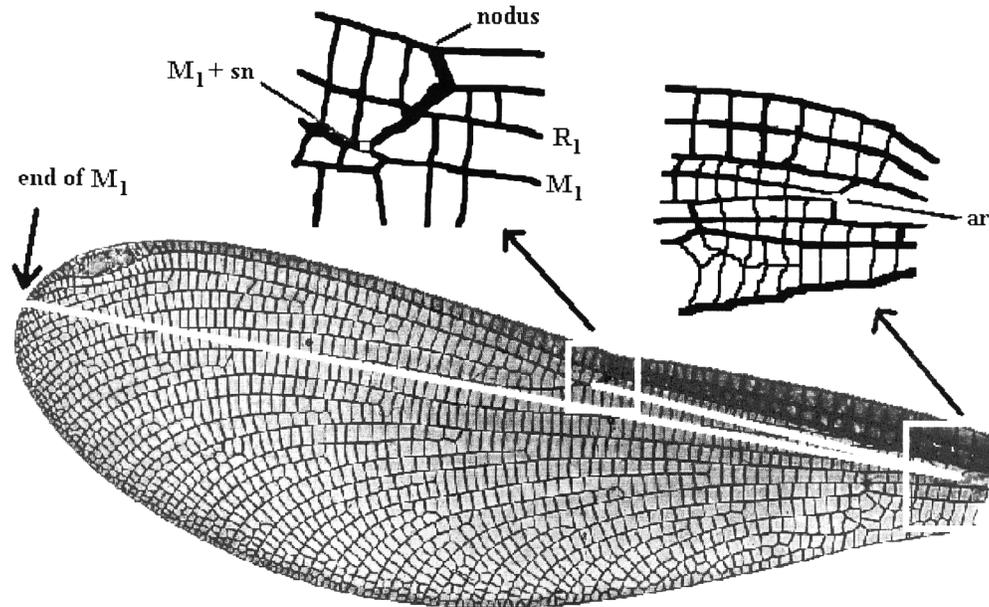


Table 1. Results of a mixed-model ANOVA conducted on the wing segments ($n = 75$ for each sex) and wing lengths ($n = 42$ for each sex) of male and female *Calopteryx maculata*.

	Sex	F (ANOVA)			Effect-trait size
		Individual	Side	Individual \times side	
Segment					
Fore-wing	Male	419.06 ***	27.06 ***	12.71 ***	0.08
	Female	605.48 ***	8.20 **	14.19 ***	0.03
Hind-wing	Male	559.94 ***	10.23 **	16.33 ***	0.19
	Female	499.06 ***	5.31 *	18.36***	0.26*
Length					
Fore-wing	Male	239 061.0 ***	4.67 *	15.15***	0.02
	Female	3 644.2 ***	11.74 **	35.44***	-0.21
Hind-wing	Male	3 041.5 ***	1.44	28.11***	-0.24
	Female	3 339.6 ***	0.09	53.61***	0.32*

Note: Significance of "individual" indicates that the differences between individuals are larger than the measurement error; significance of "side" indicates the presence of DA; significance of "individual \times side" interaction indicates that FA is larger than the measurement error. "Effect-trait size" refers to the correlation coefficient (r) between trait size ($0.5 \times$ left + right) and unsigned asymmetry (the absolute value of left minus right) (cf. Windig and Nylin 1999). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; values not footnoted are not significant.

1998). Variance in unsigned asymmetry was compared among the sexes and sites using F tests on the absolute values of left-wing minus right-wing values (i.e., Levene's test; Palmer and Strobeck 1992). For each trait, we evaluated the effect of site and sex on mean signed asymmetry, using two-way ANOVAs. To determine whether there were allometric effects on magnitudes of asymmetry, we calculated Pearson's product-moment correlation coefficients between trait sizes and asymmetries.

Results

Measurement error

Mixed-model ANOVAs conducted on the full (for segments) and subsample (for lengths) data sets verified that

measurement error did not contribute significantly to any forms of asymmetry (Table 1). The tests for the combined scanning-effect (multiple scans and flip-side scans) and human-measurement error resulted in variance components of less than 0.1% for each. The asymmetry values calculated from the flip-side scans were not significantly different from those calculated from the original scans (paired t test, $P > 0.2$). Thus, we are confident that our data were not affected by the various potential sources of error.

Testing for normality

Skewness and kurtosis measures revealed that asymmetry distributions were symmetrical, and no evidence of platykurtosis

Table 2. Mean segment size of left and right fore wings and hind wings (\pm SE), mean of left-wing segment minus right-wing segment (\pm SE), t statistic (tested against zero), and unsigned directional asymmetry as a percentage of within-group mean segment size for *C. maculata* sampled from three sites.

Site	Left (mm)	Right (mm)	Difference (mm)	t	% difference
Male fore-wing segment					
Forsyth	12.946 \pm 0.099	13.037 \pm 0.107	-0.092 \pm 0.036	-2.51*	1.17
Peterson	13.336 \pm 0.070	13.455 \pm 0.083	-0.119 \pm 0.025	-4.72***	0.97
Black	12.891 \pm 0.108	13.000 \pm 0.105	-0.109 \pm 0.044	na ^a ***	1.42
Female fore-wing segment					
Forsyth	14.085 \pm 0.112	14.096 \pm 0.110	-0.012 \pm 0.029	-0.41	0.79
Peterson	14.196 \pm 0.098	14.270 \pm 0.100	-0.074 \pm 0.019	-4.01***	0.69
Black	14.065 \pm 0.104	14.138 \pm 0.116	-0.073 \pm 0.043	na ^a	1.27
Male hind-wing segment					
Forsyth	11.414 \pm 0.096	11.452 \pm 0.083	-0.038 \pm 0.031	-1.21	1.04
Peterson	11.781 \pm 0.065	11.862 \pm 0.077	-0.081 \pm 0.023	-3.57**	0.99
Black	11.369 \pm 0.086	11.402 \pm 0.086	-0.033 \pm 0.022	-1.52	0.8
Female hind-wing segment					
Forsyth	12.396 \pm 0.086	12.384 \pm 0.099	0.011 \pm 0.032	0.35	1.1
Peterson	12.517 \pm 0.075	12.592 \pm 0.085	-0.076 \pm 0.025	-3.07**	0.89
Black	12.387 \pm 0.085	12.426 \pm 0.089	-0.040 \pm 0.029	-1.38	0.93

Note: *, $P < 0.025$; **, $P < 0.005$; ***, $P < 0.001$ (two-tailed tests).

^aWilcoxon's rank sum test used in lieu of t test, owing to leptokurtic distributions.

was found. Some groups exhibited slight leptokurtosis in a single trait but, in each case, this was due to mild outliers (these were treated accordingly within the analyses).

Types, magnitudes, and consistency of asymmetry

The mixed-model ANOVAs revealed significant DA in the fore- and hind-wing segments of both males and females, when data for individuals from each site were pooled ($n = 75/\text{sex}$) (Table 1). When segment asymmetry was assessed on a group-by-group basis (mixed-model ANOVAs not shown; t tests are summarised in Table 2), male fore-wing segments exhibited significant DA at all sites, while female fore-wing segments exhibited significant DA at Peterson only. Hind-wing segment DA was significant for both sexes at Peterson but was not significant for samples from Black or Forsyth. Importantly, the mean asymmetry values for 11 of the 12 groups were in the same direction: right-wing segments were longer than left-wing segments (binomial test, $P = 0.003$). The magnitudes of segment asymmetry as a percentage of mean trait size ranged from 0.69% (fore-wing segments of females from Peterson) to 1.42% (fore-wing segments of males from Black) (Table 2).

Fore-wing lengths were also directionally asymmetric, when considering entire subsamples of males and females ($n = 42/\text{sex}$) (Table 1). Mixed-model ANOVAs conducted on a site-by-site basis revealed significant length FA within both males and females, over and above measurement error, within both fore and hind wings (total of 12 ANOVAs; all individual \times side mean squares (MS)/residual MS: $F_{[13,28]} > 2.09$, $P < 0.05$). One of these groups (females from Black) exhibited significant DA within fore-wing lengths (Table 3). Within all other groups, mean asymmetry values were not significantly different from zero and were normally distributed, thus conforming to FA. The magnitudes of length asymmetry as a percentage of mean trait size were smaller than those observed in the segment asymmetries (above) and ranged from 0.24% (fore-wing lengths of males from Black) to 0.61%

(hind-wing lengths of males from Forsyth) (Table 3). Although the length asymmetries were extremely small, the mean values for 9 of the 12 groups were asymmetric in the same direction: right wings were longer than left wings (binomial test, $P = 0.053$).

Comparisons of asymmetry among groups

The variance of unsigned segment asymmetry in the fore wings of females differed significantly among the sample sites (Levene's test, $F_{[2,72]} = 4.23$, $P = 0.018$) but otherwise did not differ significantly among sites for any other group. The variance of unsigned segment asymmetry in the fore wings was significantly larger in males than in females (F test, $F_{[74,74]} = 1.58$, $P = 0.05$) but otherwise did not differ significantly among sexes for any other trait. The variance of unsigned length asymmetry for both fore and hind wings was not significantly different between the sexes.

Two-way ANOVAs (site and sex as factors) indicated that signed fore-wing and hind-wing segment and length asymmetry did not vary significantly either among sites or between the sexes ($P > 0.05$).

Correlations among trait size and asymmetry

Not surprisingly, correlations among trait sizes (segments and lengths of fore and hind wings) were significant in both males and females (Tables 4 and 5). In addition, signed segment asymmetry within the hind wings of females was significantly correlated with fore- and hind-wing segment sizes (Table 4): females with longer average fore- and hind-wing segments tended to have increasingly longer right-wing segments than left-wing segments (Fig. 2). These correlations, however, had little explanatory power (<17% variation in segment asymmetry explained by either fore-wing or hind-wing segment size). Correlations between length and segment asymmetries were nonsignificant (Table 5). Only within female fore wings were length and segment signed asymmetry significantly correlated (Fig. 3) (note that within

Table 3. Mean length of left and right fore and hind wings (\pm SE), mean of left-wing length minus right-wing length (\pm SE), t statistic (tested against zero), and unsigned directional asymmetry as a percentage of within-group mean length for *C. maculata* sampled from three sites.

Site	Left (mm)	Right (mm)	Difference (mm)	t	% difference
Male fore-wing length					
Forsyth	32.258 \pm 0.317	32.301 \pm 0.318	-0.044 \pm 0.052	-0.85	0.44
Peterson	33.379 \pm 0.188	33.380 \pm 0.185	-0.001 \pm 0.051	-0.02	0.48
Black	32.401 \pm 0.200	32.409 \pm 0.185	-0.008 \pm 0.028	-0.29	0.24
Female fore-wing length					
Forsyth	35.577 \pm 0.280	35.669 \pm 0.252	-0.092 \pm 0.043	-2.11	0.42
Peterson	36.086 \pm 0.316	36.149 \pm 0.305	-0.062 \pm 0.057	-1.10	0.48
Black	36.100 \pm 0.208	36.258 \pm 0.211	-0.158 \pm 0.057	-2.79*	0.57
Male hind-wing length					
Forsyth	31.023 \pm 0.320	31.024 \pm 0.292	-0.001 \pm 0.069	-0.02	0.61
Peterson	32.139 \pm 0.171	32.089 \pm 0.182	0.050 \pm 0.041	1.23	0.4
Black	31.023 \pm 0.175	30.968 \pm 0.178	0.055 \pm 0.037	1.49	0.36
Female hind-wing length					
Forsyth	34.257 \pm 0.266	34.262 \pm 0.257	-0.005 \pm 0.066	-0.07	0.49
Peterson	34.680 \pm 0.267	34.689 \pm 0.288	-0.009 \pm 0.055	-0.15	0.42
Black	34.718 \pm 0.204	34.682 \pm 0.200	0.035 \pm 0.060	0.6	0.54

Note: *, $P < 0.05$ (two-tailed test).

Table 4. Pearson's correlation coefficients among traits for all male and female individuals ($n = 75/\text{sex}$).

	FWSD	HWSD	FWS
Males			
HWSD	0.038		
FWS	-0.142	-0.029	
HWS	-0.073	-0.048	0.946**
Females			
HWSD	0.275		
FWS	-0.133	-0.401**	
HWS	-0.189	-0.350*	0.936**

Note: Variables are fore-wing segment signed asymmetry (FWSD), hind-wing segment signed asymmetry (HWSD), fore-wing segment size (FWS), and hind-wing segment size (HWS); *, $P < 0.05$; **, $P < 0.01$ (sequential Bonferroni-adjusted probabilities).

Table 5, this correlation was not significant at Bonferroni-adjusted α levels).

Finding relatively consistent DA within the wing segments but not within the wing lengths prompted us to test the idea that the wings experience "compensatory variations" (Schultz 1926), whereby the development of the distal portions of the wings compensates for the differences in left wing and right wing segment lengths (at least along the M_1 vein; Fig. 1). If this is the case, then t tests comparing the absolute values of size-standardised length and segment asymmetries (i.e., $(L - R)/\text{mean size}$) should indicate that length asymmetries are significantly smaller than segment asymmetries, and F tests should reveal larger variances in unsigned segment asymmetries than in length asymmetries. This was indeed the case: size-standardised length asymmetry within the fore and hind wings of males and females was significantly smaller in magnitude than segment asymmetry (all four paired t tests, $df = 41$, $P < 0.005$), and so too were their variances (all four F tests, $df = 41, 41$, $P < 0.05$).

Discussion

We have provided evidence that, while the full lengths of *C. maculata* wings exhibited FA (in all but one group), a proximal lengthwise subsection of the wings (the segment) exhibited significant DA and did so in a common direction among sample groups and traits: right-wing segments were consistently longer than left-wing segments. Additional data collected on individuals sampled within Nova Scotia (Pither 1997) are consistent with these findings: 13 out of 14 groups exhibited mean right wing segment lengths that were longer than left wing segment lengths (binomial test, $P = 0.0009$) (we do not have length data for the Nova Scotia individuals). In the present study, we also found that correlations between segment asymmetry and length asymmetry within individuals were nonsignificant in all but one group (Fig. 3). These results, as well as those of the supplementary analyses conducted a posteriori (last section of Results above), support the possibility that the distal portions of the wings of *C. maculata* develop in a way that compensates for asymmetries in proximal wing components. We are unsure of the details of wing development in damselflies but, as others have pointed out (Klingenberg et al. 1998), there exist systematic left-right asymmetries in the development of internal organs within insects, and asymmetries in external traits, as observed here, may result from carry-over effects during development.

Although we found significant FA when wing lengths were considered on a site-by-site basis for each sex, it is interesting to note that, again, the mean left-right asymmetry values for each group tended to be in the direction of right-wing lengths longer than left-wing lengths (if only marginally) (Table 3). The probability that 9 out of 12 groups would have a negative mean value of asymmetry purely by chance is 5.3%. Thus, although the results of the t tests on wing lengths were not significant, there appears to be some tendency for right wings to be marginally longer than left wings. Our finding of FA within wing lengths is consistent with other (methodologically sound) studies concerning damselflies

Fig. 2. Scatterplots of hind-wing signed asymmetry within females in relation to fore-wing (a) and hind-wing (b) segment size. Scaling of the y axes is identical in the two plots. Lines represent locally weighted regressions, with smoothing parameter = 2/3. Both relationships are significantly negative (see Table 4).

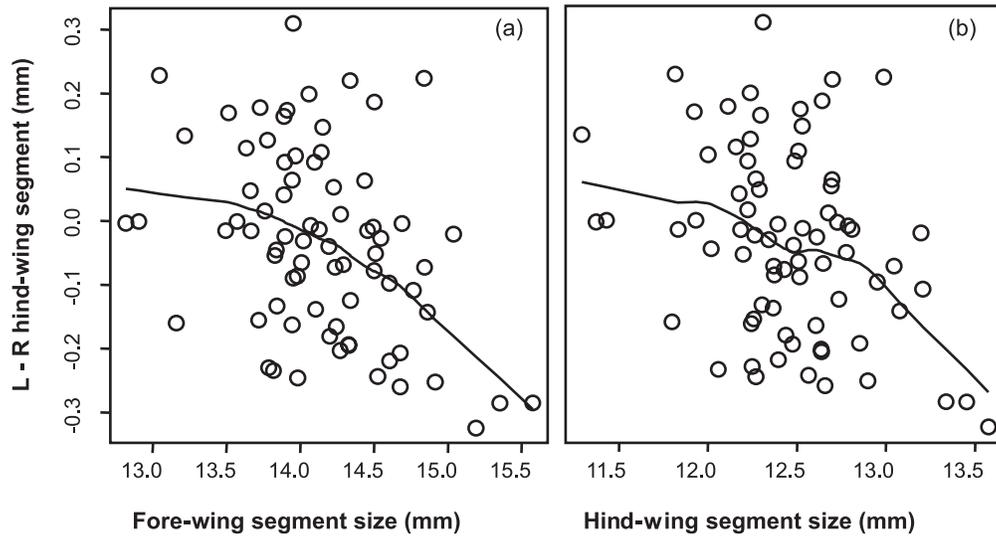


Table 5. Pearson’s correlation coefficients among traits for subset male and female individuals ($n = 42/\text{sex}$).

Trait	FWLD	FWS	HWLD	HWS	FWL	FWS	HWL
Males							
FWSD	0.176						
HWLD	0.043	-0.214					
HWS	-0.073	-0.183	0.072				
FWL	0.126	0.085	0.217	-0.072			
FWS	0.187	-0.115	0.085	-0.275	0.756***		
HWL	0.155	0.093	0.178	-0.058	0.983***	0.730***	
HWS	0.251	-0.053	0.163	-0.261	0.773***	0.945***	0.759***
Females							
FWSD	0.425						
HWLD	-0.164	-0.275					
HWS	-0.329	-0.067	0.451 ^a				
FWL	0.210	-0.048	-0.059	-0.176			
FWS	0.347	-0.011	-0.046	-0.236	0.591**		
HWL	0.220	-0.121	-0.043	-0.218	0.922***	0.588**	
HWS	0.400	-0.057	0.032	-0.301	0.642***	0.913***	0.675***

Note: Variables are fore-wing length signed asymmetry (FWLD), fore-wing segment signed asymmetry (FWS), hind-wing length signed asymmetry (HWLD), hind-wing segment signed asymmetry (HWS), fore-wing length size (FWL), fore-wing segment size (FWS), hind-wing length size (HWL), and hind-wing segment size (HWS). The two values in boldface type correspond to the significant correlations for females listed in Table 4 (for full data set). **, $P < 0.01$; ***, $P < 0.001$ (sequential Bonferroni-adjusted probabilities).

^aA single outlier renders this value significant at $p = 0.05$.

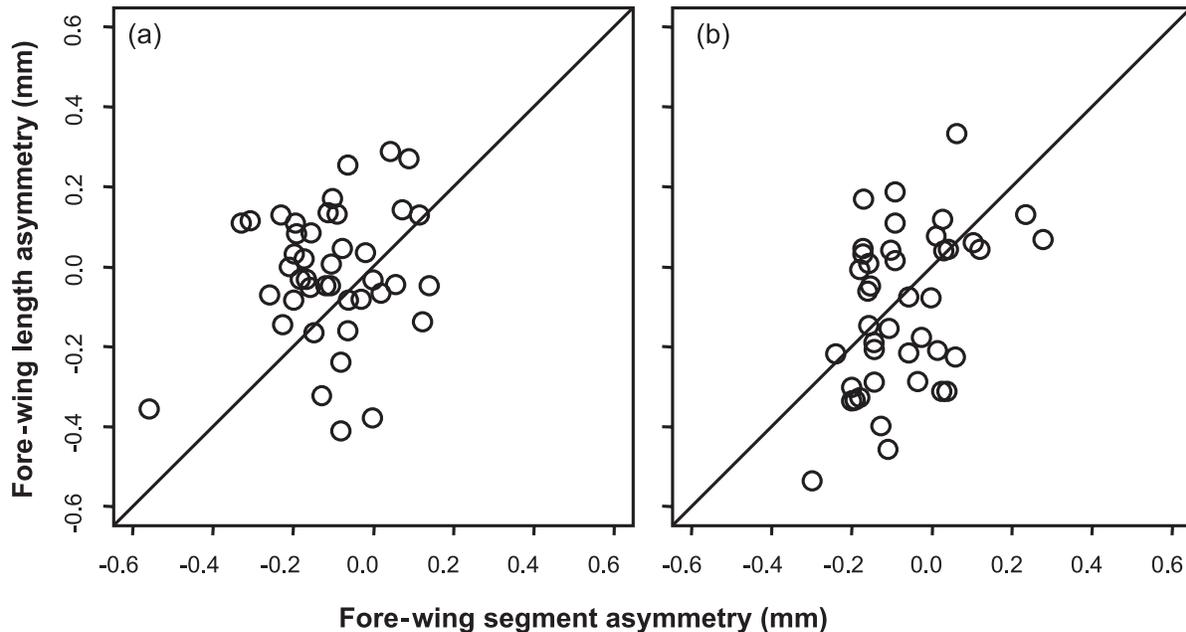
(Forbes et al. 1997; Leung and Forbes 1997; Hardersen and Wratten 1998; Hardersen et al. 1999; Carchini et al. 2000). None of these other studies, however, analysed wing traits comparable with our segments, so it remains to be seen whether DA within the proximal sections of damselfly wings is a general phenomenon.

Previous findings of DA within insects show considerable heterogeneity. Simmons and Ritchie (1996) found DA in the harps of field crickets: on average, right harps were larger than left. This result, however, involved individuals pooled from three separate populations (sample sizes 60, 39, and 26), and it was unclear whether DA was significant at each

or any of the sites when considered separately. Such consideration can be important. For example, when we pooled our length data across sample sites, fore-wing lengths of both sexes showed significant DA, each consistent in direction (Table 1). When evaluated on a site-by-site basis, length DA was nonsignificant (Table 3).

Smith et al. (1997) used procrustes methods (which analyse multiple morphometric measures simultaneously) to demonstrate the presence of DA in the wings of honeybees (*Apis mellifera*). They found that DA was more prominent in the wings of males than in females, and their measure of asymmetry involved inequalities in wing-vein placement, which was

Fig. 3. Scatterplots of fore-wing length asymmetry versus fore-wing segment asymmetry for 42 males (a) and 42 females (b). Diagonal lines represent 1:1 concordance. For males, $r = 0.176$, $P > 0.05$; for females, $r = 0.425$, $P = 0.005$. Hind-wing correlations were not significant.



somewhat similar to our segments. Using measures of mean centroid size, male tsetse flies (*Glossina palpalis gambiensis*) were shown to have larger right wings than left (Klingenberg and McIntyre 1998). Otronen (1998) found the same pattern in the small claspers of the fly *Dryomyza anilis*. The pattern is less clear for the house fly, *Musca domestica*; using procrustes methods, Klingenberg et al. (1998) found that right wings were larger than left wings (mean centroid size), while Goulson et al. (1999) used a length index similar to our segments and found that left wings were significantly longer in this trait within both reared and wild individuals (and both sexes) originating from a single site. In *Drosophila melanogaster* (a species that has been the subject of many FA studies), it has been shown that left wings are more often larger than right, but significance levels varied among samples (Klingenberg et al. 1998). Windig and Nylin (1999) found that fore- and hind-wing areas and fore-wing widths were directionally asymmetric within male but not within female speckled wood butterflies; on average, right wings had larger trait sizes than left wings. Our findings are consistent with Windig and Nylin (1999) and Smith et al. (1997) in that trait DA was more pronounced, on average, within males than within females (both concerning DA and variance in asymmetry).

Based on these few methodologically rigorous studies involving insects, it seems that, when present, DA is inconsistent in direction and significance among species and, also, within species among populations. Our findings do, however, suggest a common direction for at least segment asymmetry within *C. maculata* and, perhaps, also for length asymmetry.

One feature that is shared by the aforementioned studies is the use of high-precision measurement methods. The reported magnitudes of DA are typically extremely small, and thus, require the use of such methods to be detected. For example, we found that average differences in right- and left-

wing segments and lengths were typically less than a tenth of a millimetre in magnitude (Tables 2 and 3). This range is comparable with findings elsewhere (Klingenberg et al. 1998; Otronen 1998). As others have pointed out (Rowe et al. 1997; Goulson et al. 1999), such a stringent requirement for precise measurement techniques has likely led to a misleadingly low detection rate (and, consequently, report rate) of forms of asymmetry other than FA. Shortcomings in measurement techniques would more likely be manifested as FA than DA (Rowe et al. 1997). We carefully verified that neither systematic nor random errors influenced our measurements. We are thus confident that the patterns of segment and length asymmetry revealed here (and in the data from Nova Scotia) are real, not artifacts.

To our knowledge, only Leung and Forbes (1997) and Hardersen et al. (1999) have reported evidence for DA in damselfly wing lengths. The former study found DA in the hind wings of teneral female *Enallagma ebrium* (Hagen). The trait measured was a segment from the nodus to the pterostigma (i.e., the portion of the wing distal to our segments). Because this was the only sample out of four assessed that exhibited DA, they attributed their finding to chance, and retained all their results for subsequent FA-quality assessments. Interestingly, for a similar detection rate (5 of 16 tests), Hardersen et al. (1999) chose to discard their directionally asymmetric measures of wing lengths on similar grounds. We suspect that this latter procedure is common in FA research, and suggest that this too could contribute to an under-representation of DA within the asymmetry literature. Other studies examining asymmetry in damselfly wings (Harvey and Walsh 1993; Córdoba-Aguilar 1995; Bonn et al. 1996) would benefit from re-evaluation using more precise measuring methods and complete analyses.

This study demonstrated that, while the full lengths of *C. maculata* wings exhibited FA, a proximal segment measured along a prominent lengthwise vein showed consistent

DA. Supplemental analyses provided evidence consistent with the idea that the wings may undergo compensatory development, rendering total wing length asymmetry less variable than subpart asymmetry. We are unaware of any other studies demonstrating such consistency among sample groups in subtle DA of any insect-wing traits.

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