Odonatologica 26(1): 9-16 *March 1, 1997*

FLUCTUATING ASYMMETRY IN *COENAGRION RESOLUTUM* (HAGEN) IN RELATION TO AGE AND MALE PAIRING SUCCESS (ZYGOPTERA: COENAGRIONIDAE)

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Received March 2, 1996 / Revised and Accepted August 12, 1996

Recent evidence suggests that fluctuating asymmetry (FA) of characters may index either stress during development of organisms, or be related to fitness of individuals following development. The Authors tested whether wing FA of *C. resolutum* was related to damselfly age and to male pairing success. It was predicted that younger individuals should have higher FA on average as compared to older individuals if FA was related to damselfly survival. It was found that younger individuals had higher FA than older individuals over all sampling dates combined. However, this relation was due to the inclusion of one of three comparisons between pre-reproductive and mature insects, and was not a general phenomenon. Wing FA was not related to male pairing success. The results suggest that character FA can be related to fitness measures of damselflies, but not in a highly repeatable way. Data are also provided on seasonal changes in mite parasitism and body size that may relate to our finding FA--fitness relations restricted to one period of the flight season.

INTRODUCTION

Fluctuating asymmetry (or FA) refers to random deviations from bilateral symmetry (of a paired character) that are normally distributed about a mean of zero for a sample of individuals (see PALMER & STROBECK, 1986, 1992 for definitions of other asymmetry distributions such as anti-symmetry or directional asymmetry). FA is believed to arise from the interplay between developmental noise combined with an organism's ability to buffer against this developmental noise (PALMER & STROBECK, 1986, 1992; PALMER, 1994). Some researchers have suggested that FA measures should be related to the quality of individuals (or their ability to be symmetrical) and thus should be correlated with measures of condition or fitness (reviewed in WATSON & THORNHILL, 1994). Other researchers have argued that FA should correlate with environmental stress to populations (PARSONS, 1992; CLARKE, 1993). Some studies have found that character FA correlates inversely with quality or fitness, and correlates positively with stress (e.g. fitness: NAUGLER & LEACH, 1994; stress: CLARKE, 1993; POLAK, 1993). Other studies, however, have not found the predicted results which has prompted several researchers to outline problems with measurement, analysis and/or interpretation of FA values (e.g. PALMER, 1994).

Our main objective was to test whether wing FA was related to age of *Coenagrion resolutum*, or to pairing success of males. To do this, we first had to determine whether the distribution of wing asymmetries followed the distribution predicted for FA (rather than for other asymmetry distributions), and whether wing FA had to be corrected for wing size (following PALMER, 1994). We then examined whether wing asymmetry values differed between newly-emerged individuals and mature males and females. Such a result is expected if wing FA relates to survival over the pre-reproductive period. We also looked at pairing success of males because two studies have shown that wing FA relates to lifetime or to daily mating success of damselflies (*C. puella* [L.]: HARVEY & WALSH, 1993; *Ischnura denticollis* [Burm.]): CÓRDOBA-AGUILAR, 1995).

In relation to our objectives, we also tested for effects of sampling date on degree of parasitism by *Arrenurus* mites and body size of newly-emerged damselflies. Mite parasitism can change seasonally in at least one damselfly species (FORBES & BAKER, 1991). Moreover, mite parasitism is known to influence measures of adult fitness in damselflies (FORBES, 1991a, 1991b; FORBES & BAKER, 1991) and may thereby influence the likelihood of finding FA-fitness relations depending on whether lightly- or heavily-parasitized individuals are included in samples. In two studies on another coenagrionid (FORBES & BAKER, 1990; B. Leung & M. Forbes, unpubl.), body size of newly-emerged damselflies was related to survivorship. Seasonal declines in body size are widespread in temperate odonates (KOENIG & ALBANO, 1987; BAKER 1989, FORBES & BAKER, 1991), including a *Coenagrion* species (HARVEY & WALSH, 1993). Thus, any effects of mites on damselfly survivorship also could be influenced by size of damselflies. We could not test the parasitism hypothesis directly as mites are lost over time and mite numbers could not be compared between mature adults and tenerals for this species (cf. FORBES, 1991a, 1991b). Thus, our comparisons involving mean intensity of mite parasitism (and mean size) of tenerals over time (in association with emergence size of tenerals) are not tests of hypotheses per se, but rather examinations of whether relations may exist between parasitism, body size, FA and fitness of damselflies.

METHODS

STUDY SITE. – Our study site was a small permanent pond (ca 1-ha) that was oblong in shape. The pond was in an agricultural field near Edenwold, Saskatchewan, Canada (50°29'N; 104°16'W). It had limited macrophyte populations around its edge, including sparse tufts of cattails (*Typha* spp.) and sedges (*Carex* spp.).

COLLECTIONS AND MEASUREMENTS. – We collected 107 newly-emerged adults (55 ♂, 52 ♀) that had dried wings and that lacked mature colouration (hereafter, these adults are referred to as tenerals or newly-emerged adults). We stored tenerals individually in 85% ethanol on their emergence dates: 1, 10, and 19 June 1994 (or weeks 1, 2 and 3). We also collected 126 mature males (caught either paired to a female or unpaired) and 56 mature females (caught paired) and stored them in 85% ethanol. We collected these matures on 10, 19 and 24 June (weeks 2, 3 and 4). For *C. resolutum*, mite parasitism could be accurately assessed only for newly-emerged damselflies; engorged mites drop off of mature adults and it is difficult to accurately index parasitism using mite scars following detachment for this species (cf. FORBES, 1991b). Thus, we could not compare mite loads of newly-emerged and surviving adults.

For each individual, we measured forewing lengths (from the nodus to the third crossvein after the pterostigma) and head capsule widths (another index of body size, BAKER, 1989), using an image-analysis digitizer as described below. Each specimen remained in alcohol for only 7-10 days before being measured. In this way, we reduced any effect of alcohol on 'shrinking' of characters. However, we believe that this effect was non-existent because we measured characters that were rigid.

We measured characters using a digitizer sold as Agvision (Decagon Devices Inc). Forewings and heads were enlarged (10X) on a SONY Black & White monitor, and measured using the computer interface. By having a large image with clearly defined landmarks (especially for wings), we greatly reduced measurement error. Minimizing measurement error is especially important for analyses of character asymmetries, because such asymmetries could index FA which is expected to have a mean of zero and show small variance (PALMER, 1994). To further reduce measurement error for wing asymmetry, we took three measures (following YEZERINAC et al., 1992) of both wings and calculated wing asymmetry as the difference between the means of the right and left measures.

Finally, we examined wing FA in relation to wing size of damselflies in order to determine whether FA values had to be corrected for size before further analyses (cf. PALMER, 1994). Absolute FA often was not normally distributed for either tenerals or mature adults, using tests outlined above. Thus, we used transformed absolute values ($\sqrt{x} + 0.5$) to examine relations between wing FA and wing size.

RESULTS

MEASUREMENT ERROR, WING ASYMMETRY AND FA

Using a Nested ANOVA with sides of wings nested within a subsample of 30 males, we found our measurement error for wings was ca 0.23% in relation to among-individual variation in wing lengths, and ca 4.2% in relation to among-side variation in wing lengths (or among-individual variation in wing asymmetry measures).

Mean signed asymmetries for wings are shown in Table I for tenerals and mature adults, separately by sex. In all cases, the mean of the asymmetry distribution was not significantly different from zero. In addition, all distributions of signed asymmetries did not differ from normal distributions, using Kolmogorov-Smironov's test (d-values ranged from 0.048 to 0.15, $p > 0.05$). Thus, wing asymmetries indexed FA, and not directional asymmetry or anti-symmetry.

Table I

Mean signed asymmetries ± S.D. and 95% confidence intervals (CI) for wings of all samples of females and males included in this study. – [N refers to sample size. Two teneral females were damaged such that wing asymmetries could not be measured]

Age	Sex	N	Wing FA (mm)	95 % CI
Teneral	♂	55	-0.019 ± 0.177	-0.067, 0.028
Teneral	♀	52	-0.0035 ± 0.199	-0.053, 0.060
Mature	♂	126	-0.0326 ± 0.120	-0.054, 0.012
Mature	♀	56	-0.0020 ± 0.159	-0.045, 0.040

WING FA AND DAMSELFLY SIZE

We found that distributions of transformed FA values were not significantly different from normal distributions. For mature adult and teneral males, transformed wing FA was not related to length of the right wing (r=-0.07 and -0.03, respectively; $p > 0.05$). For teneral females, transformed FA was inversely related to length of the right wing (r=-0.41, $P < 0.01$), whereas for mature females, transformed FA was not related to length of the right wing (r=-0.03, $p > 0.05$). Although wing FA was inversely related to wing size in one instance (at $p < 0.01$), there were no instances in which wing FA was positively related to damselfly size. For this reason, it was unnecessary to correct wing FA for wing size in subsequent analyses.

WING FA IN RELATION TO AGE OVER ALL SAMPLES, AND AFTER CONTROLLING FOR TIME OF SEASON

Table II

Comparisons of absolute wing FA in mm for tenerals and mature adults, by week of emergence period. Tenerals emerging in one week are compared to mature adults collected several days later. – [For example, the comparison weeks 1-2 refers to tenerals collected in week 1 compared to adults collected in week 2]

Weeks	Teneral versus Adult		t-value	df	p
	Wing FA	Wing FA			
		Females			
1-2	0.11	0.11	-0.05	30	0.95
2-3	0.17	0.056	-2.06	29	0.04
3-4	0.11	0.13	0.41	41	0.68
		Males			
1-2	0.11	0.10	-0.41	47	0.67
2-3	0.17	0.08	-3.58	54	0.0007
3-4	0.13	0.10	-1.61	49	0.11

When we compared mature adult and teneral FA, or examined FA in relation to male mating success, we used Levene's tests on untransformed absolute FA. Absolute wing FA was related to age for males ($F_{1,179}=11.43$, $p < 0.001$), but not for females ($F_{1,104}=1.15$, $p = 0.28$). Mean absolute wing FAs ± S.D. were the following: 0.096 mm ± 0.075 mm versus 0.14 mm ± 0.10 mm (for mature versus teneral males, respectively) and 0.10 mm ± 0.12 mm versus 0.13 mm ± 0.15

mm (for mature females versus teneral females, respectively). Thus, teneral males showed higher FA on average than mature adult males over all collection dates combined which was expected if high FA individuals suffer high mortality. This result did not hold for females.

We further tested if the relation found between wing FA and male age was consistent over the season. To do this, we compared tenerals caught in week 1 with matures collected in week 2; tenerals collected in week 2 with matures collected in week 3; and tenerals collected in week 3 with matures collected in week 4, using t-tests. In so doing, matures were more likely to be compared to samples of tenerals that belonged to their cohort. Male tenerals had significantly higher wing FA than mature males for only one of three comparisons (Tab. II). Teneral females also had significantly higher FA than mature females for that same comparison (Tab. II).

Table III

Mean numbers of *Arrenurus* mites (transformed $\log_{10}(x+1)$) ± 1 S.D. for teneral males and females by week of collection. Back-tranformed means (B-tr) are also indicated. – [Post-hoc refers to post-hoc pairwise comparisons using Tukey's test and examining significant variation in mean numbers of mites across weeks of sampling. Weeks with the same letter are not significantly different from one another with respect to mean mite numbers combined for the sexes]

Week	Sex	N	Mean	SD	B-tr	Post-hoc
1	♂	16	1.33	0.32	20.4	a
	♀	15	1.13	0.54	12.5	
2	♂	20	1.25	0.22	16.8	a
	♀	17	1.43	0.24	25.9	
3	♂	19	1.14	0.24	12.8	b
	♀	20	0.97	0.46	8.3	

MITE PARASITISM AND BODY SIZE IN RELATION TO WEEK OF COLLECTION

Week of collection was significantly related to the mean numbers of mites carried by tenerals, but there was no effect of sex of tenerals on mite numbers (date: $F_{2,101}=6.14$, $p < 0.01$; sex: $F_{1,101}=0.78$, $p > 0.05$; interaction: $F_{2,101}=2.32$, $0.1 > p > 0.05$). Using Tukey post-hoc comparisons, we found that tenerals collected in week 3 had significantly lower numbers of mites than tenerals collected in weeks 1 ($p < 0.05$) and 2 ($p < 0.01$) (see Tab. III for absolute numbers of mites separately by sex and date of emergence).

Table IV

Mean wing lengths and head capsule widths (in mm ± SD) by week of emergence for teneral males and females. – [N refers to sample size]

Week	Sex	N	Wing length	Head width
1	♂	16	10.92 ± 0.38	3.75 ± 0.11
	♀	15	12.17 ± 0.36	3.75 ± 0.11
2	♂	20	10.66 ± 0.38	3.67 ± 0.11
	♀	17	12.21 ± 0.58	3.81 ± 0.17
3	♂	19	10.48 ± 0.49	3.72 ± 0.098
	♀	20	11.82 ± 0.39	3.83 ± 0.097

For tenerals, wing length was related to both sex ($F_{1,99}=254$, $p < 0.001$) and week of emergence ($F_{2,99}=7.5$, $p < 0.001$). The interaction between sex and week of emergence did not explain significant variation in wing lengths ($F_{2,99}$=1.00, p=0.37). Thus, wing length declined for both males and females throughout the emergence period with females being larger on average than males (Tab. IV). Qualitatively similar results were obtained using head capsule widths (Tab. IV), except that there was a significant interaction between sex and week of emergence in accounting for variation in mean head widths ($F_{2,100}$=3.11, $p < 0.05$).

WING FA AND MALE PAIRING SUCCESS

Finally, we examined whether mated males had lower wing FA than unmated males, based on samples across the entire flight season and by comparing samples, controlling for week of emergence. We found that 60 mated and 66 unmated males had statistically indistinguishable mean wing FA (0.10 mm ± 0.073 mm versus 0.086 mm ± 0.077 mm, respectively) when all samples were combined ($F_{1,124}$=2.67, p =0.10). Using a 2-way ANOVA, we again found no effect of either week of emergence ($F_{2,120}$=1.00, p=0.36) or mating status ($F_{1,120}$ = 2.16, p=0.14) on mean wing FA of males.

DISCUSSION

The most salient findings of this study are threefold. First, wing asymmetry was a good measure of FA because the distributions of wing asymmetry values were statistically indistinguishable from normal distributions with a mean of zero and because wing asymmetry was measured with low error. This measurement error is low for studies of character asymmetries (PALMER 1994) but it is very similar to measurement error on wings of *Enallagma ebrium* (B. Leung & M. Forbes, unpubl.). Second, wing FA was not positively related to wing size, and thus wing FA could be compared directly to measures of fitness. And finally, there was limited evidence that wing FA related to fitness measures for *C. resolutum* (i.e., there was no relation with male pairing success, and the relations with male and female ages were each restricted to one of three comparisons over the flight season). Importantly, the probability of 2 of 6 tests being significant in the predicted direction at $p < 0.05$ was 0.03 (using binomial expansion). Thus, we feel that this "seasonal effect" is real (especially given that one result was significant at $p < 0.001$).

From these results, we argue that character FA-fitness relations may be found for damselflies, but such relations are not repeatable within individual species (this study) or between species (cf. HARVEY & WALSH, 1993; CÓRDOBA-AGUILAR, 1995). What follows below is a brief discussion of what might account for discrepancies between our study and other studies.

The first obvious difference between studies is sample size. Our study was based on 181 males and 108 females collected over four sampling periods (tenerals and matures combined). For the comparison of male pairing success in relation to FA, our study was based on 60 paired and 66 unpaired males. Harvey & Walsh's study was based on 493 males of which 197 never mated over their lifetime. In comparison, Córdoba-Aguilar's study was based on 46 paired and 49 unpaired males. Thus, our study is comparable to Córdoba-Aguilar's with respect to sample size, whereas both studies have far fewer individual males represented than reported by Harvey & Walsh.

There was no attempt in any study to examine the influence of parasites on finding FA-fitness relations. Nor has there been any attempt to determine whether wing FA relates to 'flight performance' in damselflies, e.g., by affecting mate searching ability or foraging efficiency. Other studies have shown FA-fitness relations for characters associated with performance (reviewed in PALMER, 1994). If wing FA relates to flight performance, then high FA individuals that are also heavily-parasitized may suffer greater mortality. By similar reasoning, body size has been related to damselfly survival during bouts of food deprivation (FORBES & BAKER, 1990). High FA individuals of small size might be expected to die sooner when challenged by food deprivation. Currently, experiments are needed to test these ideas.

Following this reasoning, we might have expected FA-age relations to be restricted to the first sampling periods, if mite parasitism influenced the likelihood of finding such relations (due to seasonal declines in intensity of mite parasitism, with tenerals collected in week 3 having the lowest numbers of mites). By comparison we might have expected FA-fitness relations to be restricted to the last sampling period if body size influenced likelihood of finding such relations, because of seasonal declines in body size as indexed by wing length. In this study, FA-age relations were restricted to the second sampling period when mites numbers were still high on tenerals of intermediate sizes. Whether this 'seasonal effect' was due in part to mite parasitism could not be tested using our data, but our data do suggest that such tests should be conducted across samples (ranges in mite parasitism and body size are unlikely to be large within samples).

Finally, our finding that wing FA was not related to mating success of males is important because such relations have been found for two other species of damselflies using similar techniques (HARVEY & WALSH, 1993; CÓRDOBA-AGUILAR, 1995). Importantly, the magnitude of wing FA in all three studies is comparable (ranging from 0.06 mm to 0.17 mm in Córdoba-Aguilar's study, from means of 0.04 mm to 0.25 mm extrapolated from fig. 1c in Harvey & Walsh's study and from 0.056 mm to 0.17 mm in this study). We currently do not know why some studies find a relation whereas others fail to find such relations. However, it is important to publish neutral results to determine the degree to which FA-fitness relations are repeatable for specific groups of organisms using specific traits.

ACKNOWLEDGEMENTS

This study was supported by a NSERC operating grant M.F. We thank R.L. BAKER, and M. POLAK for helpful comments and T.R. MILLER for help with field and laboratory work.

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