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Variation in fluctuating asymmetry among nine damselfly species

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Fluctuating asymmetry (FA), measured as random deviations from bilateral symmetry, likely results from developmental disturbances by internal or environmental stresses. However, comparisons of FA in single damselfly species (Odonata: suborder Zygoptera) from stressed environments have often been inconclusive. We measured levels of FA among multiple species of damselflies from the same environment to determine the relative roles of environmental stress and species-specific developmental instability. Damselflies of nine species were collected from a central Texas wetland. Calculations of their FA were based on cell counts of four clearly defined areas (venation patterns) in fore and hind wings. Significant FA of venation occurred in both sexes, both wing positions, and in each of four venation patterns of all nine species. Levels of FA were not significantly different between sexes or between wing positions for any of the nine species. However, FA varied significantly among the four venation patterns. Patterns with lowest mean cell counts had significantly higher FA than the other patterns, despite scaling to remove size bias. More broadly, a three-fold difference in overall FA occurred among the nine species and was not correlated with species mean weight or abdomen length. The wide range of FA levels among multiple species in the same environment calls for caution in designing studies that select a single species expected to be sensitive to environmental stress. Future research must examine the relative roles of species-specific predispositions for FA from internal genetic stresses as well as external stressors.

Keywords: fluctuating asymmetry; environmental stress; Odonata; damselflies; developmental instability; wing venation

Introduction

The pervasiveness of bilateral symmetry in animals reflects the apparent strength of selective pressures preserving this highly conserved trait. It follows that asymmetry is sometimes maladaptive and may reflect stress during development (Bonn et al., 1996; Cornelissen & Stiling, 2010). Three asymmetries include antisymmetry, directional asymmetry, and fluctuating asymmetry. Antisymmetry describes significant and consistent difference between the magnitudes of one or more features on each side, and the larger side varies randomly among individuals. For example, the larger signaling claw of male fiddler crabs is equally frequent on both the right and left sides in nearly all related species (Palmer & Strobeck, 1986). Directional asymmetry describes characters differing on each side with the larger side generally constant, as in the coiling of gastropods (Palmer, 1994; Palmer & Strobeck, 1992; Van Valen, 1962). Fluctuating asymmetry (FA) describes random deviations between values of right and left traits of normally bilaterally symmetrical organisms (De Block et al., 2008; Palmer & Strobeck, 1986).

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Fluctuating asymmetry is of particular interest as an indicator of developmental instability (Brown et al., 2008; Leamy & Klingenberg, 2005; Soulé, 1979). Developmental instability refers to variation around the target phenotype expected from a specific genotype in a specific environment. It results from genetic or environmental stressors that disturb the development of structures along their normal developmental pathway and produce developmental "noise" (Albarrán-Lara et al., 2010; Leamy & Klingenberg, 2005). Structures on either side of a plane of symmetry are produced by the same genes, therefore asymmetrical structures likely result from deviations or accidents during development (Jagoe & Haines, 1985). A core premise underlying FA as a measure of developmental instability is that both sides of an organism are independent outcomes of the same developmental blueprint. The more bilateral symmetry an organism exhibits, the more successfully its genome has stabilized development in the face of inherent genetic or environmental stress (Parsons, 1990). FA results from either a genome that cannot adequately buffer genetic stressors during development, or from environmental stressors that overwhelm the genome's buffering capacity (Dongen, 2006; Lens et al., 2002). These causes of FA are not mutually exclusive, and act in concert. However, one may be more influential than the other. From either cause, FA may be a useful measure of the regulatory capacity of a developmental system (Carter et al., 2009; Valentine et al., 1972).

Various studies have linked FA to factors other than environmental and genetic stressors. The impact of stressful metamorphosis from larval to adult stages in a complex odonate life cycle remains unclear. However, Campero et al. (2008) suggest that stress due to metamorphosis may weaken or eliminate any use of measurements of adult FA as a reflection of larval conditions. FA can result from external disturbances such as parasite loads as well as thermal, nutritional, and pollutant stress during development (Bonn et al., 1996; Chang et al., 2007; Hardersen, 2000). The value of studying FA arises in part from its relationship to the orderly expression of genotypes as complex, three-dimensional phenotypes (Palmer & Strobeck, 1986). Many studies cite FA as an indicator of fitness and developmental stability (Bonn et al., 1996; De Block et al., 2008).

Use of FA as a tool to monitor water pollution has yielded varied results. Hardersen (2000) investigated whether life history and behavior compromise the validity of FA as a bioindicator of water pollution. Populations of the damselfly *Xanthocnemis zealandica* (McLachlan) were randomly sampled from defined groups of adults (e.g. mating and non-mating males at a breeding site, females at a breeding site) at four sites. Two sites were polluted and two were controls. Males were significantly less asymmetrical than females. The results testing levels of FA in polluted sites versus controls were ambiguous. Of the four hypotheses tested, significant results were found for only two. Chang et al. (2007) tested the effects of pesticide on FA levels of damselfly larvae and found that FA increased with insecticide use for only one of seven traits measured.

Attempts to relate FA to fitness or fitness surrogates have also been inconclusive. Beck and Pruett-Jones (2002) related FA to reproductive success, territoriality, and relative survivorship in the damselfly *Calopteryx maculata* (Beauvois). FA significantly correlated with mating status in males. However, body size rather than FA enhanced survivorship and the ability of males to defend resources. De Block et al. (2008) examined developmental costs of rapid growth in the damselfly *Lestes viridis* (Vander Linden). Their analysis suggested that females, but not males, with higher growth rates had more wing asymmetry (De Block et al., 2008). While Bonn et al. (1996) found a significant correlation between FA of forewing length and ectoparasitic water mite load, Yourth et al. (2002) found that immune response to parasitism related to season but not to FA. Bots, Van Dongen, et al. (2009) examined differences in developmental instability and fecundity between female morphs of *Enallagma cyathigerum* (Charpentier). Gynomorphs (females colored differently from conspecific males) were more asymmetrical and produced a higher mean clutch size than that of andromorphs (females colored similarly to conspecific males). Bots, Breuker, et al. (2009) found little variation between morphs when comparing wing length, wing loading and total wing surface.

The best traits for assessing asymmetry are likely those most closely associated with survivorship and fitness and therefore most practically relevant to assessing variation in natural populations. In aerial animals, wing design strongly influences survival and fitness since activities such as foraging, predator avoidance and courtship behavior depend on efficient and precise flight (Bots, Breuker, et al., 2009; Corbet, 1999). Symmetrical individuals fly more effectively due to improved flight mechanics (De Block et al., 2008; Wakeling, 1997) Wing cell counts, with their inherent number of stabilizing cross veins, were chosen for our analyses. See the review by Wakeling (1997) for a discussion of relevance for wing venation, cross veins, diverging vein patterns, and rigidity of wings as they relate to flight dynamics. Our experimental design assumes that cell counts are a direct surrogate for number of cross veins, and that these cross veins directly relate to the rigidity and therefore aerodynamics of wing function.

Leamy and Klingenberg (2005) present a thorough review of the research on the roles and interaction between environment and genetics that may promote FA. The majority of studies have used FA of multiple traits to quantify organism-wide developmental instability as a measure of individual quality or exposure to stress (Leamy & Klingenberg, 2005). It should be possible to use a combined index for FA in multiple traits to increase the precision of the estimate of organism-wide developmental instability (Leamy & Klingenberg, 2005; Lens et al., 2002; Leung et al., 2000). To this end, our experimental design incorporates a combination of four wing venation patterns to arrive at a singular assessment of FA. Inherent in this approach is the recognition of some traits being more or less sensitive to overall assessment.

If FA accurately reflects an organism's ability to produce a consistent phenotype when exposed to environmental stress, then the level of FA should be directly proportional to the amount of the stress. This proportional relationship in a range of species would constitute good evidence that FA resulted from environmental stressors (Jagoe & Haines, 1985). Conversely, a wide range in levels of FA in a number of species in the same environmental conditions could indicate genetic causes. Studies of damselflies relating FA to fitness have included larvae and adults of no more than one species or genus in each study. No studies of damselflies were found that have compared multiple species from the same environment. This study is designed to investigate the presence and levels of FA across multiple species of damselflies in the same environment. An inconsistent level of FA among species in the same environment could emphasize genetics as the cause of marked increases in FA.

Methods

Adult damselflies were collected at the Lake Waco Wetlands (central Texas, 31°36′36″ N, 97°18′28″ W). The Lake Waco wetland at McLennan Co. is an appropriate sampling area within the geographical range of the nine species described in this study. Any collected species within 170 km of the edge of that species distribution (Abbott, 2011) was eliminated to avoid interpretation of development of a species at the stressful margins of its overall distribution.

Water for the Lake Waco Wetland flows from the Bosque River. Water is pumped from the Bosque River through this 180-acre wetland and drains into Lake Waco. See Scott et al. (2005) for water quality and nutrient information for this wetland. Collections were made between 1000 and 1300 hours for 60 to 90 minutes between 15 May 2009 and 6 November 2009. No species was collected within two months of the beginning or end of their flight season (Abbott, 2007). The sampling period for collection of each species ($>90\%$ of n) was less than one summer generation, and mean daily water temperature varied no more than $\pm 4°$C. Seasonal variation during the collection period for each species was minimal for a subtropical Texas summer. To minimize variation among samples from the aquatic habitat affecting development, all captures were within a 50-m length of the shoreline bounded on each end by a further 40 m of shoreline with consistent
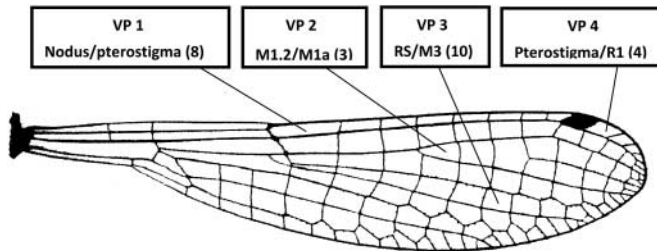
Figure 1.   Venation pattern (VP) cells counted to estimate levels of fluctuating asymmetry (FA) in forewings and hind wings of the damselfly species listed in Table 2. The figures in parentheses are the number of cells in the wing shown.

vegetation and littoral zone characteristics. Throughout the 130-m shoreline and littoral zone the depth contours, sediment type, and density of macrophytes were consistent. The slow but constant flow of water ($<0.1 \, \mathrm{m \, min^{-1}}$) through the wetland minimized differences in water quality within the sampled area. All damselflies were captured within five meters of the shoreline. All nine species have been collected at the sampling site within four years previous to this research. Tenerals were found for all sampled species, further indicating a local breeding population. In addition, the nearest local sites greater than two acres with similar damselfly communities were more than eight kilometers away, which minimizes the possibility of immigrants to our sampling site.

Males and females of available species were netted and transported to the laboratory in glassine envelopes. The damselflies were killed using ethyl acetate, weighed to the nearest 0.1 mg and identified to species. The dorsal surfaces of forewings and hind wings of each damselfly were scanned using a Canon Canoscan 8800F. Measurements of scanned specimens were made using Adobe Photoshop ® and included head width, abdomen length and meristic characters of both fore and hind wings. The four meristic characters recorded from the wings were: the number of cells from the nodus to the pterostigma (venation pattern 1), the number of cells from the branching of M1 and M2 to M1a (venation pattern 2), the number of cells between Rs and M3 (venation pattern 3), and the number of cells from the pterostigma to R1 (venation pattern 4) (Figure 1). See Bonn et al. (1996), Bots, Van Dongen, et al. (2009), Hardersen (2000), and Yourth et al. (2002) for precedents for these measured variables.

Data analysis

Initial analyses determined if asymmetry was distinguishable from background noise (Pomory, 1997). Raw measurements or counts were tested for normality. If normal, a paired *t*-test compared the mean of all higher values of each pair with the mean of all lower values. If not normal, a Wilcoxon signed-rank test compared means. Significantly different means indicated asymmetry (Pomory, 1997). The large sample size and statistical design minimized confounding measurement error terms (Carter et al., 2009). In addition, all measurements and counts were made twice.

If asymmetry was present, then the distribution of all right values and the distribution of all left values were each tested for normality. If normal, then a paired *t*-test compared the mean of right values with the mean of left values. Non-normal data were tested with a Wilcoxon signed-rank test. Significantly different means indicated directional asymmetry (Pomory, 1997).

If asymmetry was detected and was not directional, then the signed differences between the right and left sides were calculated and tested for normality. Non-normality and bimodal distributions indicated antisymmetry. Normality indicated the presence of FA (Pomory, 1997). Our initial evaluation of independent variables was based on the presence/absence of FA.

Further evaluations were based on levels of FA. Indices to quantify and compare these levels of fluctuating asymmetries were calculated to investigate biological relevance of fluctuating asymmetry. Calculations of these indices included all individuals collected during the entire sampling periods for each species. Effects of time were tested for each species (ANOVA) for collection months with sample sizes ≥20. Palmer and Strobeck (1986) review commonly used indices of fluctuating asymmetry. The most sensitive indices are variances of the right minus left differences of a character.

Methods outlined by Palmer and Strobeck (1986) compare the degree of FA for one population with that of a comparable population (e.g. asymmetry of VP1 counts for males versus counts for females). Similarly sized characters were compared with a non-scaled, more sensitive index. See Palmer and Strobeck (1986) Index 5.

$$\text{Non-scaled index of asymmetry} = \frac{\Sigma (A_i)^2}{N} \tag{1}$$

where $(A_i) = |R_i - L_i|$, $R_i = $ right side value, $L_i = $ left side value, and $N = $ number of individuals in a sample.

FA for some characters may be biased by character size (Campero et al., 2008). Indices that scale right minus left differences as a percentage of total value allow comparison of asymmetry between small and large species and between small and large characters.

$$\text{Scaled index of asymmetry} = \left(\frac{\text{var } A_i}{(R_i + L_i)/2} \right) \tag{2}$$

F-tests compared scaled or non-scaled indices of FA to reveal if FA of one population significantly differed from FA of another population.

To determine if one venation pattern exhibited greater FA than the others, multiple variances were compared using Levene's tests. Significant heterogeneity of variances among venation patterns would indicate greater FA in one or more patterns.

To summarize differences among species, we ranked each of nine species by their overall degree of asymmetry. To do so, a population asymmetry parameter (PAP) (see R_j as calculated by Soulé, 1967) was calculated. Within each species, the scaled index of FA values was calculated for each of four characters in the fore and hind wings. These values were listed by species and ranked from one to nine, with one indicating the lowest asymmetry value and nine the highest asymmetry value. The rankings were summed across the four venation patterns for fore and hind wings to determine the R_j value for each species. A high R_j value indicates high overall occurrence of FA for the species.

Results

Collections made between 15 May 2009 and 6 November 2009 at the Lake Waco Wetlands yielded 1443 damselflies in three families, five genera and 15 species. Of these, nine species (357 females, 775 males) in three genera of Coenagrionidae were deemed to be in high enough density to indicate a suitable habitat for asymmetry calculations. The smallest number collected of any species was 65 and the greatest was 357 (Table 1). Species collected but not included in data analyses due to small sample size (<9) were *Argia moesta* (Hagen), *Argia apicalis* (Say), *Argia translata* (Hagen in Selys), *Argia leonorae* (Garrison), *Lestes disjunctus* (Selys), and *Hetaerina americana* (Fabricius).

Initial analyses (see the "Data Analysis" section) indicated that FA was widespread among collected damselflies. FA was detected in all species, both sexes, both wing positions and in all

Table 1. Types of asymmetry present among damselfly species by sex, venation pattern (VP) and wing position (fore and hind).

Species	Sex	N	VP 1		VP 2		VP 3		VP 4	
			Fore	Hind	Fore	Hind	Fore	Hind	Fore	Hind
Argia sedula	F	114	FA	FA	FA	FA	FA	DA	FA	FA
	M	233	FA	FA	FA	DA	FA	FA	FA	FA
Argia nahuana	F	45	FA	FA	FA	FA	FA	FA	FA	FA
	M	142	FA	FA	FA	FA	FA	FA	FA	FA
Ischnura ramburii	F	62	FA	FA	FA	FA	FA	FA	FA	FA
	M	56	FA	DA	FA	FA	FA	FA	FA	FA
Ischnura hastata	F	30	FA	DA	FA	DA	FA	DA	NA	NA
	M	67	FA	FA	FA	FA	FA	DA	FA	NA
Ischnura posita	F	45	FA	FA	FA	FA	FA	FA	FA	FA
	M	51	FA	FA	FA	FA	FA	FA	FA	FA
Enallagma civile	F	36	FA	FA	FA	FA	FA	FA	FA	FA
	M	53	FA	FA	FA	FA	FA	FA	FA	FA
Enallagma basidens	F	25	FA	FA	DA	FA	FA	FA	FA	FA
	M	46	FA	FA	FA	FA	FA	FA	FA	FA
Argia immunda	F	2	—	—	—	—	—	—	—	—
	M	70	FA	FA	FA	FA	FA	FA	FA	FA
Telebasis salva	F	8	—	—	—	—	—	—	—	—
	M	57	FA	FA	NA	FA	FA	FA	FA	FA

Notes: FA: fluctuating asymmetry; DA: directional asymmetry; NA: no asymmetry; —: insufficient sample size for calculations.

four venation patterns (Table 1). Tests for the effects of time showed no significant differences across months of collection for any of the nine species [ANOVA, minimum F = 1.453, $p = 0.23$, df = (3, 92); max. F = 0.277, $p = 0.84$, df = (3, 98)].

Venation patterns (VP) (Figure 1) varied in levels of FA. Levene's tests revealed significant heterogeneity of scaled variances among the four venation patterns (F = 24.9, $p = {<}0.001$, df = 3). VP 2 (M1,2/M1a) and VP 4 (pterostigma/R1) had significantly higher variances ($p = {<}0.001$) than VP 1 and VP 3 and thus highest levels of FA. Mean cell counts were 9.9 for VP 1, 2.7 for VP 2, 11.9 for VP 3, and 4.1 for VP 4. The two venation patterns with the highest FA values also had the lowest mean cell count.

Comparisons of FA between forewings and hind wings yielded no significant differences. Variances (scaled index of FA) were calculated for each of the four venation patterns in fore and hind wings. Of the 52 paired variances examined, 32 were higher for VPs in forewings; 20 were higher in hind wings. A binomial expansion indicated no significant deviation ($p = 0.13$) from 50:50 ratio of differences. For corroboration, F tests determined that 18 of the 52 paired comparisons were significantly different between wing positions. Of these differences, 12 showed higher FA in forewings, and six showed higher FA in hind wings. A binomial expansion indicated no significant deviation from 50:50 ratio of differences in frequency of FA in fore versus hind wings ($p = 0.24$).

The level of FA did not differ between sexes. Variances of all venation patterns were calculated for both sexes of seven species. Of the 47 paired variances examined, 27 were higher for females and 20 were higher for males. A binomial expansion indicated no significant difference ($p = 0.38$) from a 50:50 ratio of differences. For corroboration, F tests determined 11 significant differences among the 47 paired variances. Of these differences, seven indicated a greater level of FA in males and four indicated a greater level of FA in females. A binomial expansion indicated no significant deviation ($p = 0.55$) from a 50:50 difference in frequencies of FA between sexes.

FA was present in all species studied, however the level of FA varied (Figure 2). The scaled indices for each venation pattern at each wing position for each species were ranked and R_j values were calculated (Table 2). The species with the highest R_j value exhibited the highest level of FA.
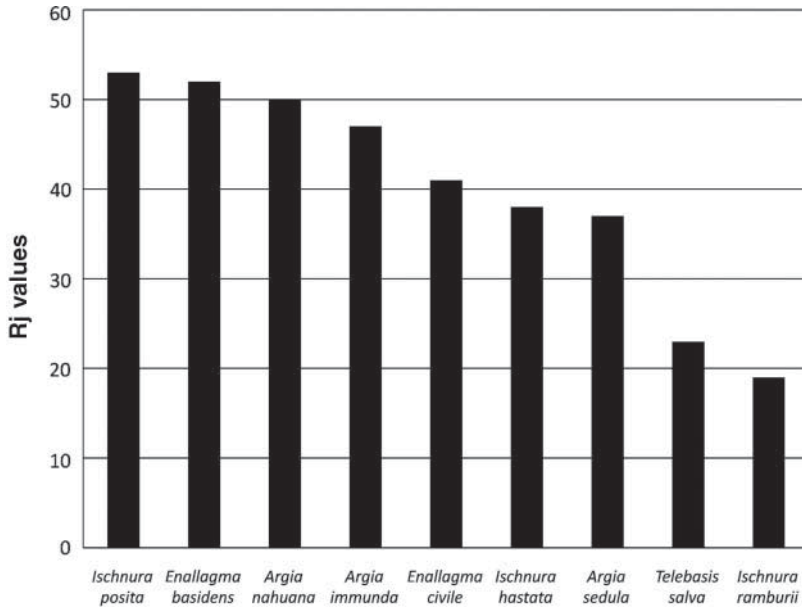
Figure 2. R_j values of each damselfly species arranged in descending magnitude to illustrate levels of FA among species.
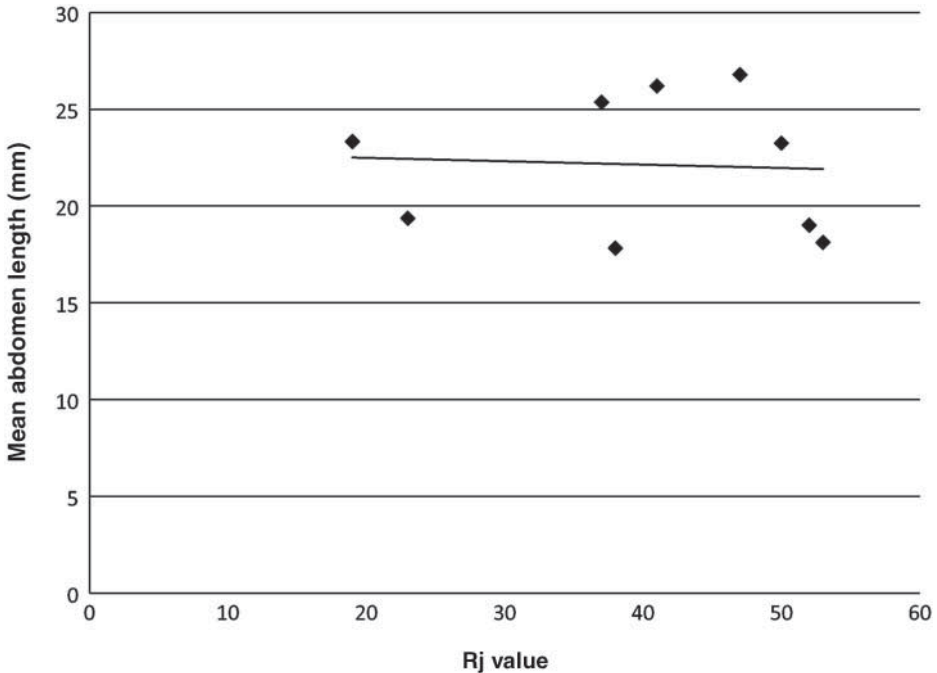

Figure 3.   Mean abdomen lengths of nine species of damselflies in relation to R_j value ($r = 0.06$).

R_j values were plotted against both mean abdomen length (Figure 3) and mean specimen weight (Figure 4). The largest species did not exhibit more FA (higher R_j values) than the smaller species either by weight or by abdomen length. Although FA varies among species, the variation is not associated with genus or correlated with mean abdomen length or with mean specimen weight.

Table 2.   Scaled indices of FA in damselflies and their ranks across four venation patterns (VP) and two wing positions (fore and hind). Scaled indices are ranked within each column and the ranks are shown in parentheses. Ranks for each species across all venation patterns are summed as R_j values.

Species	VP 1		VP 2		VP 3		VP 4		R_i
	Fore	Hind	Fore	Hind	Fore	Hind	Fore	Hind	
I. posita	0.0044	0.0050	0.0780	0.0529	0.0047	0.0030	0.0456	0.0521	53
	(7)	(7)	(5)	(6)	(7)	(5)	(8)	(8)	
E. basidens	0.0060	0.0082	0.0860	0.0439	0.0034	0.0038	0.0713	0.0493	52
	(9)	(8)	(6)	(2)	(3)	(8)	(9)	(7)	
A. nahuana	0.0038	0.0047	0.0868	0.0680	0.0048	0.0035	0.0315	0.0339	50
	(2)	(6)	(7)	(9)	(8)	(7)	(5)	(6)	
A immunda	0.0039	0.0032	0.0869	0.0586	0.0592	0.0032	0.0350	0.0264	47
	(5)	(2)	(8)	(8)	(9)	(6)	(6)	(3)	
E. civile	0.0049	0.0034	0.0650	0.0457	0.0030	0.0043	0.0284	0.0920	41
	(8)	(4)	(3)	(3)	(2)	(9)	(3)	(9)	
I. hastata	0.0043	0.0083	0.1108	0.0497	0.0039	0.000	0.0219	0.0000	38
	(6)	(9)	(9)	(5)	(6)	(1)	(1)	(1)	
A. sedula	0.0038	0.0033	0.0727	0.0581	0.0035	0.0027	0.0389	0.0301	37
	(3)	(3)	(4)	(7)	(4)	(4)	(7)	(5)	
T. salva	0.0039	0.0044	0.000	0.0476	0.0016	0.0021	0.0301	0.0262	23
	(4)	(5)	(1)	(4)	(1)	(2)	(4)	(2)	
I. ramburii	0.0032	0.0029	0.0428	0.0204	0.0037	0.0025	0.0252	0.0271	19
	(1)	(1)	(2)	(1)	(5)	(3)	(2)	(4)	

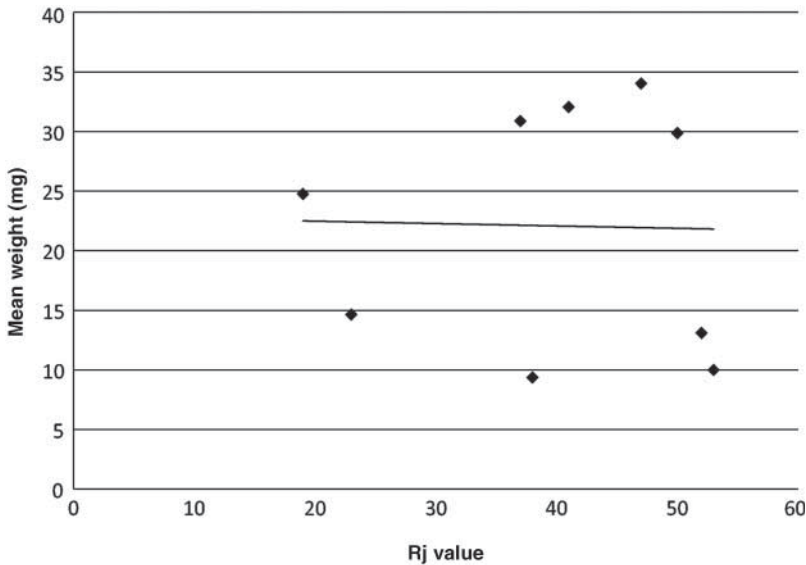

Figure 4.   Mean weight (mg) of nine species of damselflies in relation to R_j value as an overall measure of FA ($r = 0.025$).

Discussion

Random deviations of bilaterally symmetric traits, known as FA, have long been used to measure developmental instability in populations (Møller & Swaddle, 1997). Right and left sides share the same genotype, and likely are influenced by the same environmental factors. However, developmental "noise", i.e. instability, frequently arises independently on each side and results in FA. The eventual level of FA in a character depends on how successfully developmental processes reduce or buffer this "noise" (Zakharov & Yablokov, 1990) especially when challenged by environmental

stresses. Genetic and environmental stresses are both commonly cited as causes of FA; however studies show inconsistent results (Campero et al., 2008; De Block et al., 2008; Palmer & Strobeck, 1986). Unfortunately, previous studies of damselflies examining developmental instability under various stressful (environmental) conditions have used only one species. Our results comparing multiple species of damselflies from the same environment revealed considerable variation in FA not associated with broad, habitat-wide variation in environmental stress.

FA occurred in all four venation patterns (nine species), both fore and hind wings (nine species), and both sexes (seven species) studied at the Lake Waco Wetlands. This site provided a reasonably consistent environment with nutrients within average to above average ranges (Scott et al., 2005) and should isolate inherent developmental instability as the primary cause of FA. Our finding of frequent and wide ranging levels of FA across species developing in the same environment casts doubt that levels of FA are necessarily indicative of environmental conditions (stress).

Levels of FA did not vary significantly between forewings and hind wings, indicating that either wing position could provide FA data without bias. Damselfly forewings and hind wings are generally equal in length and similar in shape, although color patterns may vary (Merritt et al., 2008; Triplehorn & Johnson, 2005; Westfall & May, 2006). The significance of stable wing structure for maneuverability in pursuit of food or mates raises the probability of strong selection pressure for symmetry and developmental stability. However, FA was frequently detected and showed no difference between wing positions in these weak fliers.

Damselfly males and females showed no significant difference in occurrence or levels of FA. Jackson (1973) found small significant differences but did not analyze the sexes separately. Campero et al. (2008) and Chang et al. (2007) used larvae to study FA and sex was not determined in their studies. Bonn et al. (1996) found FA related to ectoparasitism by water mites equally in females and males. In contrast, De Block et al. (2008) and Hardersen (2000) found differences in FA levels between the sexes in some but not all drying and pesticide treatments, respectively. We found no evidence that non-territorial male pond damselflies exhibited stronger selection pressure for symmetrical wings than did the females.

FA levels among species would be expected to vary slightly as a result of differences in the genomes of each species. We found that FA, as measured by R_j values, spanned a three-fold difference among nine species (three genera). This difference was not correlated to specimen weight or abdomen length, nor was it genus specific. If FA was a good indicator of environmental conditions, then the levels of FA should be reasonably similar among species used to evaluate the same habitat. The large span in FA values found among these nine species casts doubt on its usefulness as a monitor of environmental conditions, especially conditions before metamorphosis (Campero et al., 2008). Our data reflect the probable role of inherent genetic instability, but do not fully reconcile the roles of environment versus genetics. Outcomes of these concurrent forces remain problematic (Leamy & Klingenberg, 2005).

The manifested level of FA in a mature character depends on the intensity of the stresses and how successfully the genome buffers stress. The nature and extent of the genetic basis of a genome's inherent developmental stability and its interplay with an environment's stressful challenges are not fully understood. This knowledge is essential to properly using FA as a measure of developmental instability and the impact of environmental stress (Leamy & Klingenberg, 2005). A better understanding of the genetic architecture underlying FA should explain unexpected or contradictory patterns of differences in FA levels between populations.

Conclusion

This investigation of FA among multiple damselfly species in a central Texas wetland demonstrated that FA was widespread, but its levels vary. Neither sex nor wing position predicted the magnitude

of FA. In contrast, FA among the two venation patterns with the lowest mean cell count exhibited the highest levels of FA. Finally, a three-fold difference in FA occurred among the nine species collected from an apparently unstressed wetland. Since FA can be caused by a variety of internal or environmental stresses, our study across multiple species casts doubt on the use of FA as a reliable indicator of environmental stress. Further studies demonstrating the influence of internal stresses on FA are needed to help determine appropriate use of FA as a bioindicator.

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