

ABSTRACT

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 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 casts doubt on the effective use of FA of a single species to indicate of 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

Variation In Fluctuating Asymmetry Among Nine Damselfly Species
(Odonata: Suborder Zygoptera)

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A Thesis

Approved by the Department of Biology

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Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

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May 2012

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ACKNOWLEDGMENTS

My advisor, Dr. Darrell Vodopich, helped tremendously with the field collections at the Lake Waco Wetlands and with the lab work at Baylor University. Darrell also spent a great amount of time guiding me through multiple revisions of the manuscript. Dr. Robert Baldrige and Dr. Joe Yelderman both provided helpful comments on my proposal and feedback on my thesis. Financial support was provided by Baylor University.

CHAPTER ONE

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 and others 1996; Cornelissen and 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 and Strobeck 1986). Directional asymmetry describes characters differing on each side with the larger side generally constant as in the coiling of gastropods (Van Valen 1962; Palmer and Strobeck 1992; Palmer 1994). Fluctuating asymmetry (FA) describes random deviations between values of right and left traits of normally bilaterally symmetrical organisms (De Block 2008).

Fluctuating asymmetry is of particular interest as an indicator of developmental instability (Soule 1979; Leamy and Klingenberg 2005; Brown and others 2008). 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” (Leamy and Klingenberg 2005; Albarrán

and others 2010). 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 (Jago and 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 stress or environmental stress (Bonn and others 1996). 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. 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 (Valentine and others 1972).

Various studies have linked FA to factors other than environmental and genetic stressors. The stress of metamorphosis from naiadal to adult stages in a complex odonate life cycle may result in FA (Campero and others 2008). FA can result from external disturbances such as parasite loads as well as thermal, nutritional, and pollutant stress during development (Chang and others 2007; Hardersen 2000; Bonn and others 1996). The value of studying FA arises in part from its relationship to the orderly expression of genotypes as complex, three-dimensional phenotypes (Palmer and Strobeck 1986). Therefore, many studies cite FA as an indicator of fitness and developmental stability (Bonn and others 1996; DeBlock and others 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 and others (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.

Use of FA to indicate fitness has also been inconclusive. Beck and Jones (2002) related FA to reproductive success, territoriality, and relative survivorship in the damselfly *Calopteryx maculate* (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 and others (2008) examined developmental costs of rapid growth in the damselfly *Lestes viridis* (Vander Linden). Their analysis suggested that females, not males, with higher growth rates had more wing asymmetry (De Block and others 2008). While Bonn and others (1996) found a significant correlation between FA of forewing length and ectoparasitic water mite load, Yourth and others (2002) found that immune response to parasitism related to season but not to FA. Bots and others (2009b) 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 and others (2009a) found little variation between morphs when comparing wing length, wing loading and total wing surface.

Asymmetry is calculated using a variety of metrics for traits affecting wing functionality including wing length and wing cell counts. In aerial animals, wing design strongly influences fitness and survival since activities such as foraging, predator avoidance, and courtship behavior depend on efficient and precise flight (Bots and others 2009a). Symmetrical individuals fly more effectively due to improved flight mechanics (De Block and others 2008). Wing cell counts were chosen for our analyses and correlate with wing stability, strength and elasticity (Yourth and others 2002; Bonn and others 1996; Hardersen 2000).

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 (Jago and 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 naiads 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.

CHAPTER TWO

Methods

Adult damselflies were collected between May and November, 2009 at the Lake Waco Wetlands (central Texas, 31°36'36" N, 97°18'28" W). Water is pumped from the Bosque River through this 180-acre wetland and drains into Lake Waco. See Scott and others (2005) for water quality and nutrient information for this wetland. Collections were made between 1000 and 1300 hours for 60 to 90 minutes. 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 fore wings 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 Bots and others (2009b), Bonn and others (1996), Yourth and others (2002), and Hardersen (2000) for precedence for these measured variables.

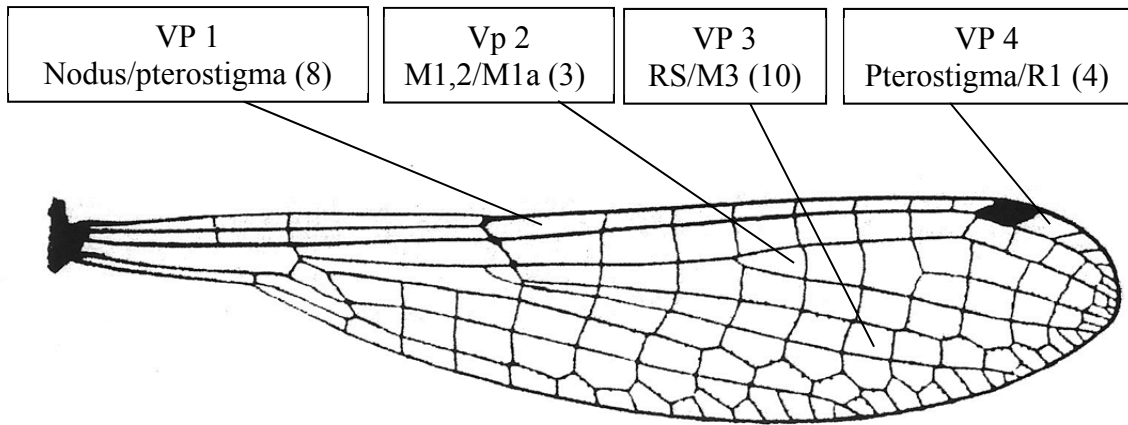


Figure 2. Venation pattern (VP) cells counted to estimate levels of fluctuating asymmetry (FA) in fore wings and hind wings of the damselfly species listed in Table 2. The figures in parentheses are the number of cells in the wing shown.

CHAPTER THREE

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 and others 2009).

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. Palmer and Strobeck (1986) reviewed 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.

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

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 and others 2008). Indices that scale right minus left differences as a percent of total value allow comparison of asymmetry between small and large species and between small and large characters.

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

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 homogeneity 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 Rj

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 relates to high overall occurrence of FA for the species.

CHAPTER FOUR

Results

Collections made between May 15, 2009 and November 6, 2009 at the Lake Waco Wetlands yielded 1,443 damselflies in 3 families, 5 genera and 15 species. Of these, 9 species (357 females, 775 males) in 4 genera of Coenagrionidae were used for asymmetry calculations (Table 1). Species collected but not included in data analyses due to small sample size were *Argia moesta* (Hagen), *Argia apicalis* (Say), *Argia translate* (Hagen in Selys), *Argia leonorae* (Garrison), *Lestes disjunctus* (Selys), and *Hetaerina americana* (Fabricius).

Initial analyses (see Data Analysis) indicated that FA was widespread among collected damselflies. FA was detected in all species, both sexes, both wing positions and in all four venation patterns (Table 1).

Venation patterns (VP) (Figure 1) varied in levels of FA. Levene's tests revealed significant heterogeneity of scaled variances among the four venation patterns ($F = 24.94$ $p = <.001$). VP 2 (M1,2/M1a) and VP 4 (pterostigma/R1) had significantly higher variances ($p = <.001$) 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 fore wings 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

Table 1. Types of asymmetry present among damselfly species by sex, venation pattern (VP) and wing position (Fore and Hind). Fluctuating asymmetry (FA); Directional asymmetry (DA); no asymmetry (NA); insufficient sample size for calculations -.

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

higher for VPs in fore wings. Twenty 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 fore wings, and 6 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 50:50 ratio of differences. For corroboration, F tests determined 11 significant differences among the 47 paired variances. Of these differences, 7 indicated a greater level of FA in males and 4 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.

The presence of FA was consistent among 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. 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.

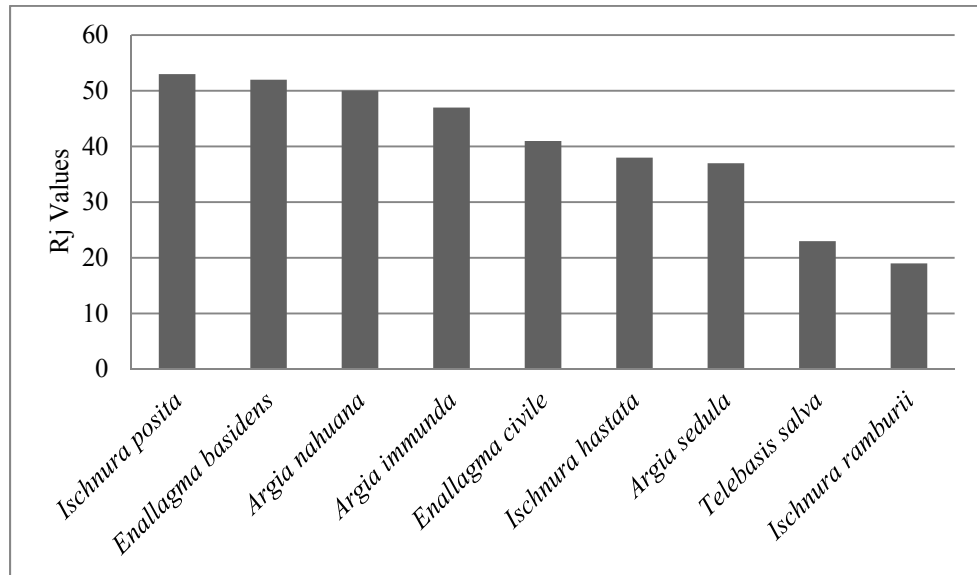


Figure 2. R_j values of each damselfly species arranged in descending magnitude to illustrate levels of FA among species.

Table 2. Scaled indices of FA in damselflies and their ranks across 4 venation patterns (VP) and two wing positions (Fore and Hind). Ranks are summed as R_j values.

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

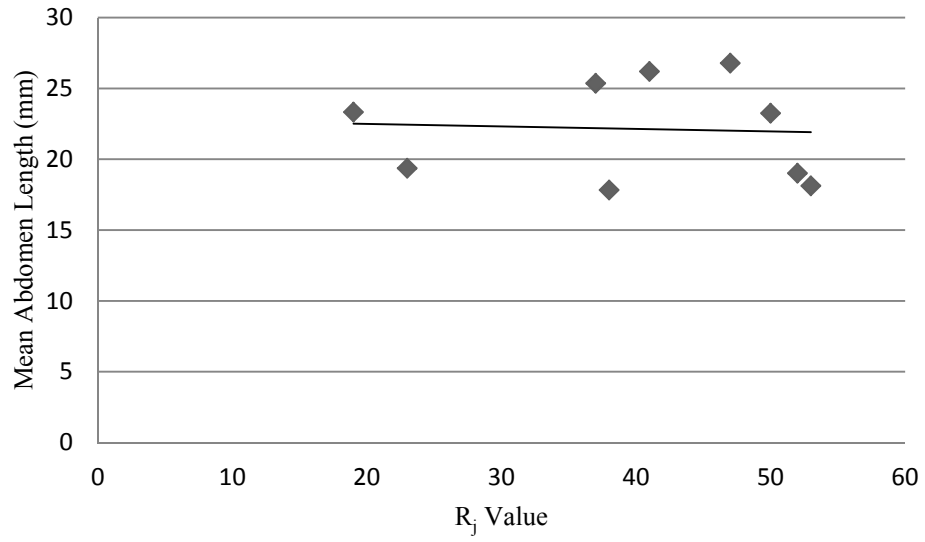


Figure 3. Mean abdomen length of nine species in relation to R_j value (r=0.06).

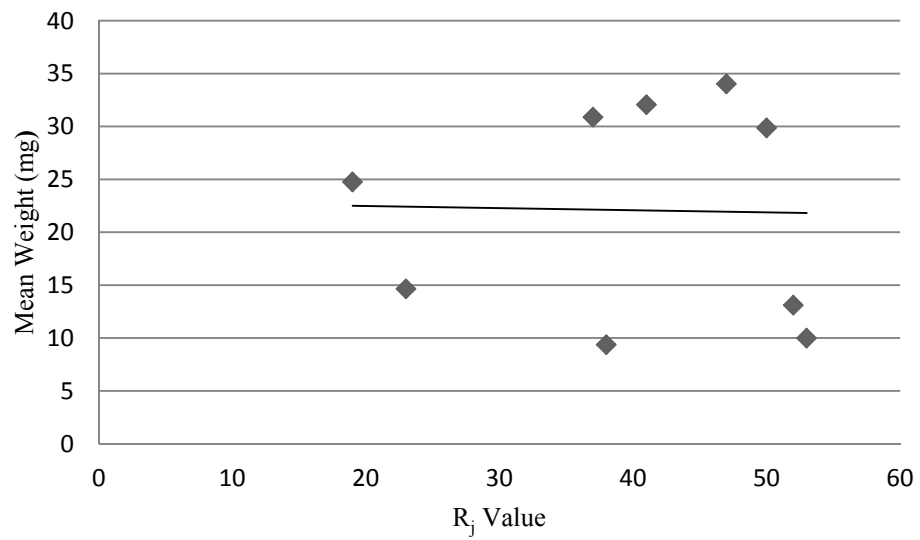


Figure 4. Mean weight (mg) of nine species in relation to R_j value (r=0.025).

CHAPTER FIVE

Discussion

Random deviations of bilaterally symmetric traits, known as FA, have long been used to measure developmental instability in populations (Møller and 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 1990) especially when challenged by environmental stresses. Genetic and environmental stresses are both commonly cited as causes of FA, however studies show inconsistent results (Block and others 2008; Palmer and Strobeck 1986; Campero and others 2008). 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 marked variation in environmental stress.

FA occurred in all four venation patterns (9 species), both wing positions (9 species), and both sexes (7 species) studied at the Lake Waco Wetlands. This site provided a reasonably consistent environment with nutrients within average to above average ranges (Scott and others 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).

Cell counts in four wing venation patterns were used to evaluate FA, and levels of FA varied among these patterns. These conserved venation patterns are often used in identification and classification (Westfall and May 2006). Two of these patterns registered consistently higher levels of FA, and therefore may be regarded as more sensitive to the causes of FA. These two venation patterns (VP 2,VP 4) had the lowest mean cell counts and had significantly higher levels of FA than the other two venation patterns (VP 1,VP 3) despite scaling to remove specimen size bias. These results indicated the choice of venation patterns to be measured may influence detection of and comparison of FA between populations.

Levels of FA did not vary significantly between fore wings and hind wings, indicating that either wing position could provide FA data without bias. Damselfly fore wings and hind wings are generally equal in length and similar in form (Merritt and others 2008; Triplehorn and Johnson 2005). 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 and others (2008) and Chang and others (2007) used naiads to study FA and sex was not determined in their studies. Bonn and others (1996) found FA related to ectoparasitism by water mites equally in females and males. In contrast, Block

and others (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 (four 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.

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 use FA as a measure of developmental instability as well as the impact of environmental stress (Leamy and Klingenberg 2005). A better understanding of the genetic architecture underlying FA should explain unexpected or contradictory patterns of differences in FA levels between populations.

CHAPTER SIX

Conclusion

This investigation of FA among multiple damselfly species in a central Texas wetland demonstrated that FA was widespread, but its levels varied. Neither sex nor wing position predicted the magnitude of FA. In contrast, FA among the two venation patterns with the lowest mean cell count indicated the highest levels of FA. This indicated that the use of a parameter more sensitive to FA would be more beneficial when investigating FA as a bioindicator. Finally, a three-fold difference in FA occurred among the nine species collected from an 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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