

Fluctuating asymmetry indicates levels of competition in an even-aged poplar clone

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Rettig, J. E., Fuller, R. C., Corbett, A. L. and Getty, T. 1997. Fluctuating asymmetry indicates levels of competition in an even-aged poplar clone. – *Oikos* 80: 123–127.

Fluctuating asymmetries are small random deviations from perfect bilateral symmetry that result from imperfect regulation of development. Recently, fluctuating asymmetry has been suggested as a tool for monitoring levels of ecological stress within and between populations. Unfortunately, such comparisons of fluctuating asymmetry may be confounded by genetic or age differences among environments. In this study we use a genetically controlled field experiment to determine differences in fluctuating asymmetry due solely to different competitive regimes. We determined fluctuating asymmetry in poplar leaf shape for leaves taken from an even-aged clone grown in six different competitive regimes. We show that increases in intra- and interspecific competition increase fluctuating asymmetry in the leaves of this clone. This supports the use of fluctuating asymmetry as a tool for detecting common environmental stresses such as competition.

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Many plants and animals have morphological structures that are bilaterally symmetrical. Deviations from bilateral symmetry may represent developmental perturbations (Van Valen 1962, Palmer and Strobeck 1986, Parsons 1990). Random deviations from symmetry, known as fluctuating asymmetry, are commonly used to detect perturbations on animals, such as the presence of toxins, abrupt inbreeding, or other “unnatural” stresses (Valentine and Soule 1973, Valentine et al. 1973, Siegel and Doyle 1975, Clarke 1992, 1993, Pankakoski et al. 1992). Although most studies have focused on animals, we use plants to see whether fluctuating asymmetry reflects the magnitude of a “natural” biotic interaction such as competition.

Deviations from symmetry within a population fall into three categories. Directional asymmetry is characterized by consistently greater trait development on one

particular side, either left or right, within the population. A good example is the mammalian heart (Van Valen 1962). Antisymmetry occurs when there is greater development of a trait on one or the other side of an individual, but the side varies randomly in the population, resulting in a bimodal or platykurtic distribution (Van Valen 1962). For example, in fiddler crabs the signaling claw is much larger but has an equal tendency to be on the right or left side (Palmer and Strobeck 1986). Fluctuating asymmetry (FA) is characterized by small random deviations from perfect bilateral symmetry, resulting in a normal distribution of the asymmetry between the two sides within a population (Van Valen 1962). FA is thought to be due to imperfect developmental stability or homeostasis resulting from genomic and environmental stress during trait ontogeny (Palmer and Strobeck 1986, Leary and Allendorf 1989, Parsons

Accepted 3 February 1997

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ISSN 0030-1299

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1990, Clarke 1992). Therefore, FA has been used as an indicator of a range of genetic and environmental stressors or perturbations that influence phenotypic quality or fitness in the lab and in the field (e.g., Valentine and Soulé 1973, Siegel and Doyle 1975, Markow and Ricker 1991, Clarke and McKenzie 1992, Møller 1992, Alados et al. 1993, Clarke 1993, Freeman et al. 1993, Naugler and Leech 1994, Nilsson 1994).

Recently, fluctuating asymmetry has been suggested as a tool for monitoring levels of ecological stress within and between populations (Leary and Allendorf 1989, Zakharov 1990, Clarke 1992). Unfortunately such comparisons of fluctuating asymmetry are usually confounded by differences in age or genetic background among environments. Therefore, differences in FA may simply be due to genetic or age differences among sites (e.g., inbreeding, hybridization, chromosomal abnormalities) and not due solely to environmental differences (e.g., pollution levels, temperature extremes, parasite load, or competition). We use a genetically controlled field experiment to determine differences in FA due solely to an extrinsic stress of different competitive regimes.

Methods

Our field experiment isolates environmental contributions to fluctuating asymmetry (FA) because the use of an even-aged plant clone controls for genetic and age differences. Therefore, differences in FA observed in the system can be attributed to our experimental manipulation of intra- and interspecific competition. These competitive regimes were established using an even-aged clone of *Populus euramericana* (cultivar Eugenei) trees planted at the W. K. Kellogg Biological Station's Long Term Ecological Research site in southwestern Michigan, USA.

Our field experiment utilized 18 LTER poplar plots, which were spatially grouped into three blocks of six plots. These blocks were established in 1989, with each block containing six 540-m² plots set up as a 3 density \times 2 weed factorial design. The three density treatments, trees planted at low (0.167/m²), medium (0.5/m²), and high (2/m²) density, create an intraspecific competitive gradient. The two weed treatments, trees planted in weedy and weedless plots, create an interspecific competitive regime. Crossing the two factors produces six treatments in each block. In weedy plots, weeds were allowed to establish naturally, except for the addition of red fescue in early 1990 as a ground cover. Weedless plots were established with a broadcast herbicide mixture in the first year, and maintained in subsequent years by a combination of hand-hoeing and application of contact herbicide to the weeds. Other studies of these poplars show that these intra- and

interspecific competitive regimes are stressful, as indicated by a significant negative impact on poplar growth. Maas (1992) found that tree diameter, tree height, leaf area, and above ground biomass all decreased with both increasing poplar density and the presence of weeds. Marino and Gross (pers. comm.) found the same decreases in growth as well as changes in branch architecture.

We quantified asymmetry in the leaves of poplars by collecting multiple samples from each experimental plot. Specifically, we collected leaves in mid-July 1995, by haphazardly choosing 20 trees in each plot and removing the outermost fully-opened leaf with no insect damage from the lowest main branch of each tree. We lost five leaves from the high density weedless plot in block 1, giving us a total of 355 poplar leaves. We examined only one leaf per tree because we were interested in characterizing the pattern of asymmetry within the plot, not within an individual tree. We measured leaves by taking the perpendicular distance to the nearest mm from the mid-rib to the right and left edge at the widest point of each leaf. Three people independently measured each leaf to allow us to check for measurement error.

Before interpreting our data, we performed the cursory statistical analyses suggested by Swaddle et al. (1994) and Palmer (1994) in order to confirm the presence of FA in each plot. These 18 plots are the experimental units (sensu Hurlbert 1984) to which the treatments were applied. Within the context of analysis of variance, trees within plots are not statistically independent if we want to address the following question: do increases in intra- and interspecific competition lead to increases in FA? This research question focuses on the effect of the treatments; therefore, we used the mean of the leaf measurements in each plot (i.e., experimental unit) to test for treatment effects. Although the analysis may be run using individual leaf measurements, with the results being very similar to the analysis of plot means, an analysis using individual leaves would be incorrect to test for treatment effects. Our cursory analyses to check for FA are detailed in the results, as is the analysis of variance on the treatment means. We used Systat (Wilkinson 1989) and SAS (SAS 1985) to run all statistical analyses and all significance tests are two-tailed.

Results

Does FA exist?

Yes, FA does exist in the poplars (see The pattern of FA, below); however, we first provide details on how we detected FA. An important initial step in analyzing asymmetry is distinguishing variance in leaf asymmetry from measurement error (Palmer 1994, Swaddle et al.

1994). In each plot, we performed a two-way analysis of variance (sides \times individual) on the leaves to determine if our measurement error (Appendix, MS_m) was smaller than the other non-directional asymmetry present in the leaves. In all cases, measurement error was significantly less than other non-directional asymmetry (p -values adjusted using a sequential Bonferroni correction). In addition, we performed an analysis of variance on a random subset of the data which shows that the repeatability of our measurements is significantly greater than measurement error ($F_{73,146} = 5.54$, $p < 0.001$, left leaf side repeatability = 98.3%, signed difference ($R - L$) repeatability = 87.4%). Given this high repeatability and our low measurement error, we averaged the 3 measurements for each leaf.

Having distinguished leaf asymmetry from measurement error, we must determine whether the pattern of asymmetry represents directional asymmetry, antisymmetry, or FA (Swaddle et al. 1994). We used two procedures to check for directional asymmetry in each plot. First we utilized the mean squares generated by the two-way analysis of variance mentioned above, to ask if the leaf sides differed in size (Palmer 1994). In all 18 plots, we found no significant difference between the two sides (p -values adjusted using a sequential Bonferroni correction), indicating a lack of directional asymmetry. Second, we used one sample t -tests on the signed differences in leaf width ($R - L$) (Swaddle et al. 1994) and found that they did not differ significantly from zero (p -values adjusted using a sequential Bonferroni correction), indicating that these differences are not directional asymmetries. To check for antisymmetry in each plot, one may examine skew and kurtosis for individual leaf asymmetry (Appendix, ($R - L$)) with the expectation that in a normally distributed population, both parameters equal zero (Palmer 1994). However, Palmer (1994) does not recommend using skew and kurtosis for smaller data sets (i.e., < 30), such as in our plots. Instead tests for non-normal distributions show that none of the 18 plots deviate significantly from normality (p -values adjusted using a sequential Bonferroni correction), indicating that our deviations from symmetry are fluctuating asymmetry rather than antisymmetry.

Improper interpretations of FA may arise if FA covaries with trait size and trait size differs among treatments (Swaddle et al. 1994). We performed linear regressions between absolute value of leaf asymmetry ($|R - L|$) and leaf size ($(R + L)/2$) for each plot (see Palmer 1994), to examine the impact of leaf size on $|R - L|$. The slope between $|R - L|$ and leaf size did not differ significantly from zero for any of the eighteen plots (Appendix, $p > 0.18$ in all cases), indicating no relationship between trait size and FA. In addition, mean leaf size for each plot does not differ among treatments (1 factor blocked ANOVA, $F_{5,10} = 2.974$, $p = 0.07$).

The pattern of FA

For our dependent variable we used the mean of the absolute value of leaf asymmetry ($|R - L|$, Appendix) (i.e., FA1, Palmer 1994) in each of the eighteen plots. This is our index of FA (Palmer and Strobeck 1986, Swaddle et al. 1994). We proceeded with a blocked 3×2 analysis of variance on our index of FA, treating density and weed factors as fixed variables and the block factor as a random variable (Table 1). We found that increases in intra- and interspecific competition were associated with higher levels of FA under the conditions of our experiment. There was a significant effect of poplar density on FA and fluctuating asymmetry was significantly higher in weedy plots (Table 1, Fig. 1). In addition, we found a significant interaction between inter- and intraspecific competitive regimes (Table 1, Fig. 1).

Discussion

Our study clearly shows that FA can be an indicator of competitive stress with the level of FA increasing as competition increases. An increase in both inter- and intraspecific competition leads to greater FA. The significant interaction of poplar density and weed level on FA shows that the effect of one stressor depends on the level of the second stressor. For example, the presence of weeds does not have an appreciable effect on FA at low poplar density. However, at higher densities of poplar clones interspecific competition increases FA. Although our goal is not to determine the mechanism behind this competitive stress, it likely involves differential limitation of resources such as nutrients, water, and light, across the inter- and intraspecific competitive regimes. Such resource limitation may stress and impair the plant's homeostatic regulating mechanisms during

Table 1. ANOVA (randomized block design) on the plot means of absolute leaf asymmetry, our index of FA. ANOVA residuals are normally distributed (Wilk's Shapiro Test W : Normal 0.958, $p = 0.558$) and the treatment variances are homogeneous (Hartley's Test $F_{max} = 7.67$, $p = 0.05$). The error term used to calculate the F value for density is the mean square (MS) of block \times density, $df = 4$. The error term used to calculate the F value for weeds is the MS block \times weed, $df = 2$. The error term used to calculate the F value for the density \times weed factor is the model error term which is the MS block \times weed \times density, $df = 4$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Source	DF	MS	F
Block	2	0.132	
Density	2	0.820	67.557***
Weeds	1	0.418	19.554*
Density \times Weeds	2	0.1573	31.834**
Block \times Density	4	0.012	2.456
Block \times Weeds	2	0.021	4.325
Error	4	0.210	

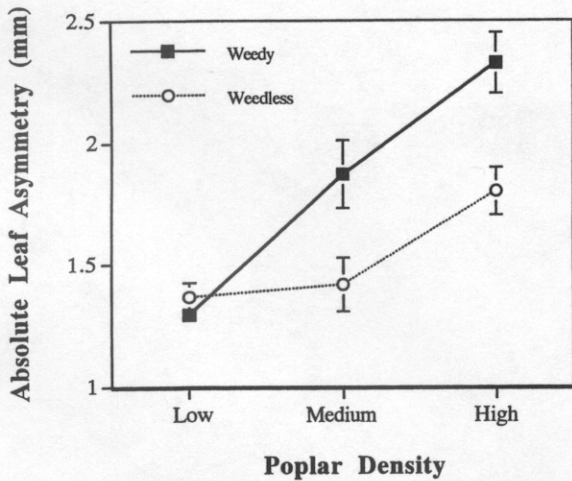


Fig. 1. Absolute leaf asymmetry (mean \pm SE) in weedy and weedless treatments is plotted for low, medium, and high densities. Weedy means: low density = 1.294 ± 0.006 , $n = 3$; medium density = 1.872 ± 0.140 , $n = 3$; high density = 2.328 ± 0.124 , $n = 3$. Weedless means: low density = 1.361 ± 0.058 , $n = 3$; medium density = 1.417 ± 0.111 , $n = 3$; high density = 1.802 ± 0.099 , $n = 3$.

growth, resulting in deviations from perfect bilateral symmetry. In fact, Nilsson (1994) manipulated resource levels available to nuthatches and found a negative relation between FA and energy intake.

Many field studies on plant and animal populations have considered the relationship between FA and abiotic stressors, such as pollutants, but only a few have considered biotic stressors (e.g. Møller 1992, Nilsson 1994). Several field studies on plant and animal populations show a strong correlation between environmental stressors and the level of FA (Valentine and Soulé 1973, Valentine et al. 1973, Clarke 1992, 1993, Pankakoski et al. 1992, Alados et al. 1993, Freeman et al. 1993, Møller 1993, 1995). Parsons' (1990) review suggests that the level of FA tends to increase as habitats become ecologically less suitable for the organisms. Although FA is a suggested method for monitoring ecological stress (Leary and Allendorf 1989, Zakharov 1990, Clarke 1992), field studies often are lacking in experimental or statistical controls for genetic or age structure differences within or among populations (but see Nilsson 1994), thus differences in FA in these studies cannot be solely attributed to the environmental stressor. By using one poplar clone, our study controls for genetic and age differences and shows that FA can be a sensitive indicator of competitive stress. Since we had access to only one clone, we are unable to evaluate whether different genetic clones may vary in their response to these competitive regimes.

Competition has long been known to influence performance parameters such as individual growth, reproduction, and survival (see reviews in Connell 1983, Schoener 1983 and Goldberg and Barton 1992). How-

ever quantifying responses of these parameters to competitive stress may involve difficulties. For example, quantifying the effects of ecological stress in terms of fecundity or survival may require a longer period of study than that necessary to quantify differences in FA. This is due to the fact that when developmental stability is disrupted by stress, changes in asymmetry can appear far sooner than changes in fecundity or survival. FA also may provide a more sensitive indicator of stress than parameters like survival or fecundity by having a measurable response at a lower threshold of stress (Clarke 1992). The ease of measurement and degree of sensitivity of FA mean it can be a convenient early warning indicator of ecological stresses and a useful tool for biomonitoring.

Acknowledgements – We would like to thank A. Tessier, L. Chen, K. Desmarais, A. P. Møller, F. W. Allendorf, E. Lyons and A. M. Jarosz for their valuable advice. Support was provided by the NSF Research Training Group at KBS (NSF grant DIR 91-13598) and the KBS LTER program (NSF grant DEB 92-11711). This is W.K. Kellogg Biological Station contribution no. 834.

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Appendix. Summary of descriptive data for the 18 subplots. Density factors (Dens.) are high (H), medium (M), and low (L). Weed factors (Weed) are weedy (+) and weedless (0). (R + L)/2 represents leafsize, (R - L) represents individual leaf asymmetry, $|R - L|$ is the absolute value of leaf asymmetry and is our index of FA (=FA1, see Palmer 1994). FA10 (σ^2 specifically) is an additional index of FA calculated using a two-way ANOVA (sides \times individual) to determine the between-sides variance once measurement error has been removed (Palmer 1994, Palmer and Strobeck 1986). Slope comes from the regression of $|R - L|$ versus leaf size.

Block	Dens.	Weed	N	(R + L)/2		(R - L)			Kurtosis	R - L		FA10 σ_i^2	df
				Mean \pm 1 SE	Slope \pm 1 SE	Mean \pm 1 SE	Skew	Mean \pm 1 SE		MS _m			
1	H	+	20	35.042 \pm 1.466	0.014 \pm 0.086	0.017 \pm 0.774	1.77	3.77	2.483 \pm 0.537	0.900	5.691	17.14	
2	H	+	20	28.192 \pm 1.066	0.062 \pm 0.061	-0.950 \pm 0.495	0.74	0.40	2.083 \pm 0.282	1.450	2.033	12.29	
3	H	+	20	34.142 \pm 2.248	0.079 \pm 0.054	-0.617 \pm 0.751	-0.79	0.82	2.417 \pm 0.541	0.617	5.439	17.64	
1	M	+	20	28.817 \pm 0.850	0.065 \pm 0.093	0.933 \pm 0.501	-0.83	1.29	1.867 \pm 0.340	0.425	2.364	16.90	
2	M	+	20	31.550 \pm 0.903	0.048 \pm 0.092	0.400 \pm 0.508	0.85	1.01	1.633 \pm 0.357	0.517	2.410	16.53	
3	M	+	20	33.292 \pm 0.925	-0.113 \pm 0.080	-0.550 \pm 0.567	-0.15	-0.94	2.117 \pm 0.333	1.025	2.870	15.13	
1	L	+	20	31.467 \pm 1.470	0.004 \pm 0.027	-0.100 \pm 0.320	-0.37	-0.79	1.267 \pm 0.167	0.450	0.908	13.93	
2	L	+	20	32.400 \pm 1.187	0.019 \pm 0.026	-0.367 \pm 0.296	0.31	-1.11	1.233 \pm 0.135	1.317	0.380	3.83	
3	L	+	20	31.208 \pm 0.914	0.005 \pm 0.057	0.483 \pm 0.349	-0.15	0.85	1.383 \pm 0.223	0.517	1.047	13.95	
1	H	0	15	42.667 \pm 2.944	-0.001 \pm 0.028	-1.244 \pm 0.501	0.03	-1.56	1.956 \pm 0.301	0.333	1.773	16.81	
2	H	0	20	33.008 \pm 1.433	0.041 \pm 0.034	-0.750 \pm 0.384	0.31	-0.53	1.617 \pm 0.212	0.692	1.219	13.36	
3	H	0	20	34.783 \pm 1.228	0.002 \pm 0.072	1.100 \pm 0.423	0.67	-0.58	1.833 \pm 0.376	2.400	0.990	5.55	
1	M	0	20	28.000 \pm 1.016	-0.037 \pm 0.071	-0.567 \pm 0.461	0.36	0.67	1.600 \pm 0.307	0.425	1.982	16.53	
2	M	0	20	28.492 \pm 0.971	0.008 \pm 0.063	-0.683 \pm 0.347	-0.68	-0.46	1.217 \pm 0.261	1.217	1.038	14.11	
3	M	0	20	28.517 \pm 0.085	0.040 \pm 0.085	-0.667 \pm 0.403	-0.71	1.25	1.433 \pm 0.283	0.508	1.456	15.21	
1	L	0	20	28.117 \pm 0.708	0.001 \pm 0.074	-0.033 \pm 0.390	0.24	-0.56	1.467 \pm 0.222	0.475	1.361	15.21	
2	L	0	20	30.350 \pm 1.050	0.011 \pm 0.040	0.033 \pm 0.267	-0.13	-0.31	1.267 \pm 0.180	0.625	0.472	8.94	
3	L	0	20	30.575 \pm 0.783	0.079 \pm 0.071	-0.350 \pm 0.385	-0.79	-0.60	1.350 \pm 0.242	0.200	1.416	17.32	