

LEAF FLUCTUATING ASYMMETRY INCREASES WITH HYBRIDIZATION AND ELEVATION IN TREE-LINE BIRCHES

BRIAN J. WILSEY,¹ ERKKI HAUKIOJA, JULIA KORICHEVA, AND MATTI SULKINOJA

Laboratory for Ecological Zoology, Department of Biology, University of Turku, Turku 20014, Finland

Abstract. Fluctuating asymmetry (FA), or random non-directional deviations from perfect symmetry in bilaterally or radially symmetrical traits, has recently been proposed to be a useful indicator of genetic and environmental stress. However, the factors underlying fluctuating asymmetry, especially in plants, remain poorly understood. We sampled leaf fluctuating asymmetry among and within three species of even-aged birch (*Betula pubescens*, *B. nana*, and *B. pendula*) and their interspecific hybrids growing in common gardens of northern Finland to determine whether hybridization and environmental stress are associated with increased developmental instability. Our predictions were that: (1) interspecific hybrids among *B. pubescens*, *B. nana*, and *B. pendula* would have enhanced leaf FA compared to the parent groups because of a possible disruption of coadapted genomes; (2) intermediates between *Betula pubescens* ssp. *tortuosa* and *B. pubescens* ssp. *pubescens* would have a slight decrease or no difference in leaf FA compared to the parent group; and (3) fluctuating asymmetry would increase between a low-elevation and a tree-line-elevation site, and this increase would be greater in *B. pubescens*, which forms the major subarctic community below the tree line, than in *B. nana*, which is common above the tree line.

As predicted, leaf FA was higher in the tree-line common garden than in the lower elevation garden, but the amount of increase was not significantly different between *B. pubescens* and *B. nana*. Leaf asymmetry was also higher in hybrids than in parental taxa, and this response was fairly consistent among the three crosses. This suggests that interspecific hybridization resulted in higher developmental instability. Furthermore, in the intraspecific studies with *B. pubescens*, there was no relationship between tree height (an intraspecific hybrid index) and leaf asymmetry. Thus, these data support the ideas of Markow (1995) that the changes in asymmetry observed from hybridization will be a function of how closely related parental taxa are. Since leaf FA in *Betula pubescens* increased with elevation and hybridization, and both are important in tree-line dynamics, FA might be a useful indicator of stress and distributional limits in this and possibly other plant species.

Key words: *Betula*; developmental instability; environmental stress; Fennoscandia; fluctuating asymmetry in leaves; hybridization; subarctic ecology.

INTRODUCTION

Fluctuating asymmetry, or random non-directional deviations from perfect symmetry in bilateral or radially symmetrical traits, is increasingly being discussed as a potential indicator of genetic and environmental stress in animals (e.g., Leary and Allendorf 1989, Parsons 1991, Palmer 1996). Since the same genes code for proteins in each half of symmetrical traits, differences between halves is a measure of developmental noise, and the organism's inability to correct for developmental noise, or developmental instability (Mather 1953, Soulé 1979, Parsons 1991, Palmer 1996). Fluctuating asymmetry (FA) is higher in some (but far from all) inbred, environmentally stressed or

hybrid populations in animals (reviewed by Palmer and Strobeck [1986], Parsons [1991], and Palmer [1996]).

Fewer studies have been conducted with plants, but increased levels of FA have been associated with pollution (Freeman et al. 1993, Kozlov et al. 1996, Kryazheva et al. 1996), inbreeding (Sherry and Lord 1996), and herbivory from leaf miners (Møller 1995). Freeman et al. (1993) found that pollution led to higher leaf FA in plants from the genera *Acer*, *Robinia*, *Convolvus*, *Aegopodium*, *Epilobium*, and *Fucus*. Kozlov et al. (1996) found that leaf FA in *Betula pendula* and *B. pubescens* decreased with distance away from sources of pollution and that the extent of increased FA was greater around sources that produced more emissions (also see Kryazheva et al. [1996]). However, the biological factors underlying fluctuating asymmetry, especially in plants, remain poorly understood (Freeman et al. 1993, Markow 1995, Sherry and Lord 1996). For example, whether heterozygosity and genomic coadaptation affect FA, and if FA is related to fitness, remains unclear (Palmer and Strobeck 1986, Markow

Manuscript received 25 March 1997; revised 26 June 1997; accepted 3 October 1997.

¹ Present address: Department of Biology, McGill University, 1205 Ave. Dr. Penfield, Montreal, Quebec, Canada H3A 1B1.

1995, Palmer 1996). Various potentially confounding effects, such as antisymmetry (lack of symmetry in normally developing leaves) and differential shading effects have also not always been taken into account (Palmer 1996). For example, in some species, leaves are typically asymmetrical, and the degree of asymmetry can change with ontogeny (McLellan 1993, 1990).

One situation in which the effects of genetic stress on FA can be assessed is in the analysis of hybrids. Hybridization is common in plants, with a large percentage of plant species formed through hybridization events (Grant 1981). Although researchers have shown that hybrid zones between plant species sometimes have increased or decreased concentrations of some herbivorous insect and fungal species (e.g., Whitham 1989, Boecklen and Spellenberg 1990, Floate and Whitham 1993, Fritz et al. 1994, Gange 1995) and are areas of potential speciation and evolution (Floate and Whitham 1993, Moorehead et al. 1993), little is known about how leaf asymmetry is affected by hybridization between plant species. Freeman et al. (1995) found little difference in 28 asymmetry characters in hybrids formed in a hybrid zone between two subspecies of *Artemisia tridentata*.

We tested the hypotheses that hybridization and elevation stress would lead to higher FA by comparing natural hybrids to pure birch trees, and by comparing *B. pubescens* trees growing in a tree-line common garden at the very margin of its distribution to trees from a lower elevation garden in Northern Finland. Our objectives were to test whether: (1) interspecific hybrids among *B. pubescens*, *B. nana* and *B. pendula* have enhanced FA compared to the parent groups; (2) intermediates between *B. pubescens* ssp. *tortuosa* and *B. pubescens* ssp. *pubescens* have a difference in FA compared to the parent group, i.e., whether the effects of hybridization on FA would depend on how closely related the parents are; and (3) FA increases between a low-elevation and a tree-line elevation site, and if this increase would be greater in *B. pubescens*, which forms the major subarctic forest community below the tree line, than in *B. nana*, which is common above the tree line.

METHODS

Birch experimental system

Three species of birch are common in Fennoscandia: (1) *Betula pubescens*, which varies from short (1–2 m) trees with many trunks/ramets in the north (subspecies *tortuosa*), to taller (>2 m) trees with fewer main trunks/ramets in the south (subspecies *pubescens*), and occurs in many parts of Europe below the tree line; (2) *B. pendula* (silver birch), which occurs commonly in Finland up to 69°20' N and is usually a tall, upright tree with usually one main trunk; and (3) *B. nana* (dwarf birch), which is restricted to moist soils (e.g., bogs) in

the south, but is much more common in the northern part of Fennoscandia and especially above the tree line; it is characterized as being a short, prostrate shrub with many ramets/trunks (Kallio and Mäkinen 1978). In central Europe and southern Fennoscandia, an efficient incompatibility system and differences in timing of flowering prevent frequent hybridization between species, but in northern Europe, this incompatibility system breaks down, and hybridization is common (Elkington 1968, Kallio et al. 1983, Eriksson and Jonsson 1986). Crosses between *B. nana* and *B. pendula*, which both have 28 chromosomes, occur in both directions, whereas crosses between *B. pubescens* (56 chromosomes) and the other two species occurs mostly from pollination by *B. pubescens* (Kallio et al. 1983, Eriksson and Jonsson 1986). Interspecific hybrids between *B. pubescens* and the other two species have 42 chromosomes (Anamthawat-Jonsson and Tomasson 1990). Introgressive hybridization between *B. pubescens* and *B. nana* is especially common in northern Fennoscandia and is thought to be responsible for the subspecies *tortuosa* (Elkington 1968, Kallio et al. 1983, Anamthawat-Jonsson and Tomasson 1990).

Experimental design and data analysis

Trees were sampled from two common gardens belonging to the University of Turku at the Kevo Research Institute in northern Finland. Common-garden birches were established by planting nursery-reared, 2-yr-old seedlings evenly spaced (1 m apart) into natural soil in 1976–1978, 18–20 yr before our sampling in the summer of 1996. The tree-line garden is at the line between birch forests and tundra (280 m above sea level [a.s.l.]); the lower garden is located in the mountain-birch forest zone (100 m a.s.l.).

For the interspecific hybridization experiment, measurements were made on trees from the lower common garden. We made measurements on 12 trees (3 randomly selected trees from each of 4 half-sib families) for each of the following groups: (1) pure *Betula pendula*, (2) *B. pendula* × *B. nana*, (3) pure *B. nana*, (4) *B. nana* × *B. pubescens*, (5) pure *B. pubescens*, and (6) *B. pendula* × *B. pubescens*. The F1 status of hybrids has been verified by chromosome counts (M. Sulkinoja, unpublished data), and in most cases F1 hybrids are readily distinguished by leaf morphology (Fig. 1), and especially by color. The hybrid groups represent the most common form of interspecific hybridization found in these birch species (Kallio et al. 1983, Anamthawat-Jonsson and Tomasson 1990). Trees were from seeds from open-pollinated parent trees from several origins: *B. pubescens*, *B. nana*, and hybrids were from around Kevo, near Utsjoki, Finland (69°45'), and *B. pendula* pure parents and hybrids from near Inari (69°1'–11' N).

Birches have two types of shoots: short shoots, which flush early in the spring and expand with resources from the previous year, and long shoots, which extend from

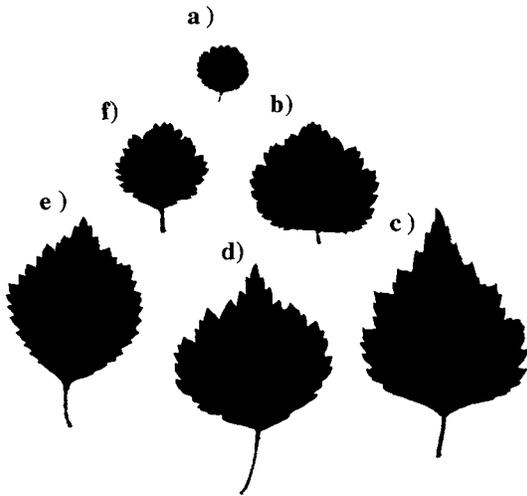


FIG. 1. Representative leaves from the following *Betula* taxa: (a) *B. nana*, (b) *B. pendula* × *nana*, (c) *B. pendula*, (d) *B. pendula* × *pubescens*, (e) *B. pubescens*, and (f) *B. nana* × *pubescens*. Leaves are actual size.

a bud in the short shoots and develop throughout the growing season with resources from the current year (Kozłowski and Clausen 1966, Haukioja et al. 1990). For leaf asymmetry, we collected 10 mature leaves from short shoots throughout the canopy of each tree (trees were short enough to sample all branches), and mounted them on herbarium paper for later measurements. For each leaf, we measured the width of the right and left halves from the midrib to the leaf margin at the mid-point (half-way between the base and tip), perpendicular to the midrib (Kozlov et al. 1996). Leaf fluctuating asymmetry was then calculated as: $FA = |L - R|/size$, where L = width of the left side, R = width of the right side, and $size = (L + R)/2$ (Palmer and Strobeck 1986). Within-plant fluctuating asymmetry, which can be reasonably high in some species (Sherry and Lord 1996), was accounted for by basing tree-wide estimates on 10 leaves per tree. Also, there is no evidence that leaf FA varies systematically among leaves within short shoots, or in specific leaves over time within a single growing season in *B. pubescens tortuosa* (B. J. Wilsey, unpublished data). Measurements were averaged across leaves within trees before statistical analyses were done (i.e., trees were the experimental units).

The presence of directional asymmetry (a consistently larger left or right side) and antisymmetry can complicate the interpretation of asymmetry as a measure of developmental stability (Van Valen 1962, Palmer and Strobeck 1986). We tested for their presence in leaves by testing $L - R$ against 0 with t tests (for directional asymmetry). We also tested for antisymmetry, which is a lack of symmetry in leaves that might have a genetic basis (e.g., McLellan 1993, 1990), with the Shapiro-Wilk test for normality of $L - R$ (SAS Institute 1988).

A second measure of developmental instability was based on variation in ramet (trunk) angles within trees. Measures of within-plant variance have also been used as measures of developmental instability in plants (Paxman 1956, Levin 1970, Bagchi and Iyama 1983, Baret and Harder 1992, and Sherry and Lord 1996). We measured the angle of each main ramet (up to five measurements per tree) by comparing the ramet to an upright ruler (90°) for each tree. Ramet angle variance (RV) was then calculated as: $RV = \sum |x - \mu|/n$, where x = observation, μ is the mean of all observations per tree, and n is the number of ramets per tree.

One-way ANOVA with a priori contrasts were used to test whether differences existed among the six *Betula* groups. An overall hybrid vs. pure contrast was made to see if there was a hybrid effect across taxa, and then the pure parents were compared to each hybrid between them for the three groups to test if a hybrid effect was found for each cross. Family was considered to be a random effect in the statistical model, and thus, family(group) was used as the error term for group effects and contrasts. For each tree, the mean FA for the 10 leaves was used in the analysis.

For the intraspecific analyses within *B. pubescens*, we included the 12 trees used in the interspecific hybridization comparison, plus 15 other preselected trees with a range of characteristics associated with the *tortuosa* and *pubescens* subspecies level (origins ranged from near Rovaniemi, Finland [$66^\circ33'$] to Utsjoki [$69^\circ45'$]). A hybrid index (Grant 1981, Moorehead et al. 1993) was created by measuring (1) tree height to the nearest centimeter, (2) number of main ramets, and (3) mean ramet angle, with “*tortuosa*-like” trees having many main ramets, being relatively short and prostrate, and “*pubescens*-like” trees having the opposite characteristics. Data from these measurements were then analyzed with principal components analysis to create a hybrid index, and the relationships between FA and the hybrid index were analyzed to determine if trees with intermediate characteristics between the two subspecies had different leaf FA compared to trees at ends of the gradient. Nested ANOVA (with variation within families as the error term) on each of the three characters was also performed to verify that the three characters had a genetic origin (i.e., that it was a legitimate hybrid index).

For the test of environmental stress on leaf asymmetry, we sampled 9 trees per taxa—3 trees from each of three half-sib families in both the upper and lower gardens for pure *B. nana*, pure *B. pubescens*, and *B. nana* × *B. pubescens* hybrids (a total of 18 trees for each taxa; all origins were from different sites around Utsjoki, Finland $69^\circ45'$). In all cases, the same families were sampled in both gardens. Sampling was performed in a similar manner to that of other data sets.

RESULTS

Interspecific hybridization

Leaf asymmetry was higher in hybrids than in parental taxa, which suggests that interspecific hybrid-

TABLE 1. Results of ANOVAs of leaf fluctuating asymmetry among three pure (*Betula pubescens*, *B. nana*, and *B. pendula*) and three hybrid (*B. pendula* × *B. nana*, *B. pendula* × *B. pubescens*, and *B. nana* × *B. pubescens*) birch taxa.

Source of variation	df	MS	F	P
Taxa	5	0.0133	7.91	0.0001
Family (Taxa)†	18	0.0017	1.03	0.4511
Error (within families)	48	0.0017		
Contrasts				
All hybrids vs. all pure	1	0.0410	24.41	0.0001
<i>B. pendula</i> × <i>B. nana</i> vs. pure <i>B. pendula</i> and <i>B. nana</i>	1	0.0081	4.85	0.0325
<i>B. pendula</i> × <i>B. pubescens</i> vs. pure <i>B. pendula</i> and <i>B. pubescens</i>	1	0.0266	15.82	0.0002
<i>B. nana</i> × <i>B. pubescens</i> vs. pure <i>B. nana</i> and <i>B. pubescens</i>	1	0.0205	12.22	0.0010

† Error term for taxa and contrasts.

ization resulted in higher developmental instability (Table 1), and this response was fairly consistent among the three crosses. Leaves of *Betula nana* × *pubescens* were approximately twice as asymmetrical as either of the parental taxa (Fig. 2). Pure *B. pendula* had higher leaf FA (fluctuating asymmetry) than did pure *B. nana* and *B. pubescens*—possibly because the common garden was 100 km north of the northern limit of the range for *B. pendula*, but well within the natural range of the other two species. Nevertheless, *B. pendula* × *B. pubescens* hybrids were much higher in leaf FA than either parent (i.e., there was a clear hybrid effect). *Betula nana* × *B. pendula* hybrids were also significantly higher than the mean of the two parents, but the difference was not as large as with the other crosses. There was no significant variation among families within taxa (Table 1).

We did not detect either directional asymmetry or antisymmetry (e.g., the asymmetry found by McLellan

1990, 1993) in either pure (*t* tests for each taxa: $0.31 < P < 0.61$; Wilks-Shapiro tests for each taxa: $0.52 < P < 0.91$) or hybrid taxa (*t* tests: $0.09 < P < 0.98$; Wilks-Shapiro tests: $0.44 < P < 0.64$). This means that the difference between left and right sides followed a normal distribution with a mean close to zero. Therefore, the asymmetries observed were primarily from fluctuating asymmetry.

Our estimate of ramet-angle variance was also greater in hybrids than in pure taxa ($F = 14.1$, $df = 5, 18$, $P < 0.001$, Fig. 3). In each of the three specific comparisons, the hybrids all had higher variation in ramet angle than the pure taxa (all contrasts, $P < 0.001$).

Intraspecific hybridization

The principal component that we created from plant characteristics (i.e., the hybrid index) accounted for 72% of the variation in the data set and created a gradient of *B. pubescens* trees that ranged from short, prostrate trees with a number of main ramets (“tor-

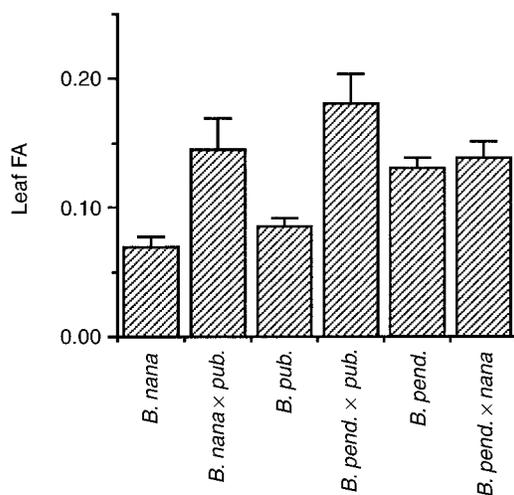


FIG. 2. Leaf fluctuating asymmetry (FA), adjusted for leaf size, in three *Betula* species and three interspecific hybrids (as in Fig. 1) in trees growing in a common garden in northern Finland. Data are means and 1 SE, for 12 trees per taxa. $FA = |L - R|/size$, where L = width of the left side, R = width of the right side, and $size = (L + R)/2$.

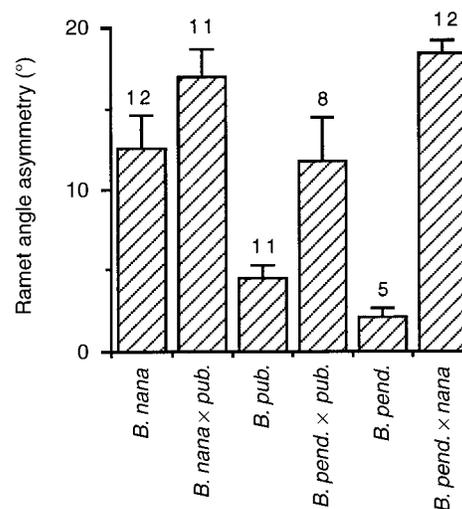


FIG. 3. Ramet/trunk angle variance in three birch species and three interspecific hybrids. Data are means and 1 SE; the numbers above histogram bars are the number of trees per group.

TABLE 2. Variables and loadings for the hybrid index within *Betula pubescens*.

Variable	PC1†
Mean ramet angle per tree	0.61
Number of ramets per tree	-0.60
Tree height	0.52
Eigenvalue	2.16
Proportion of variance	0.72

† Principal components analysis, axis 1.

tuosa-like”) to taller, upright trees with few or one main ramets (“*pubescens*-like”). Trees with *tortuosa*-like characteristics had the highest leaf fluctuating asymmetry, and leaf FA declined as trees became more *pubescens*-like ($r_s = -0.53$, $P < 0.008$; Table 2, Fig. 4a). However, tree height was the only variable for which significant genetic/maternal variation existed ($F =$ family mean square error [MSE] divided by the individual nested within family MSE; $F = 5.1$, $P = 0.023$). Since the other two variables were primarily of

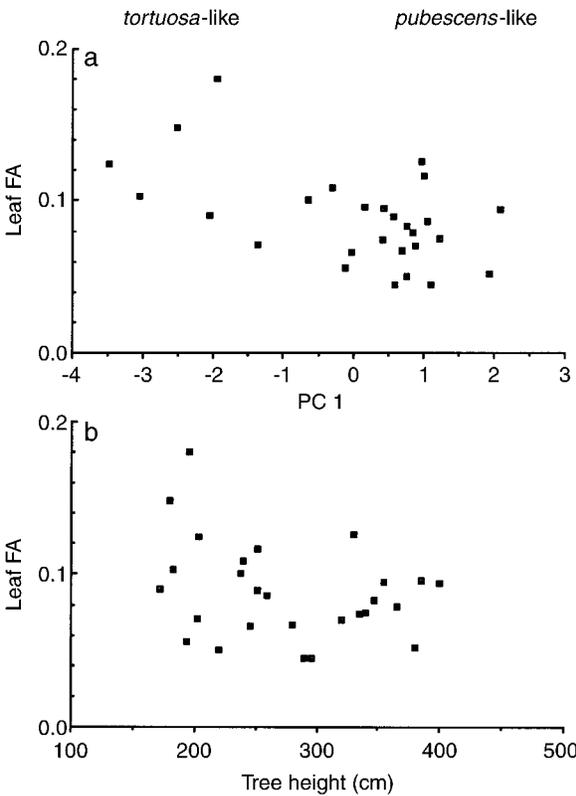


FIG. 4. Relationship between leaf fluctuating asymmetry (FA) and plant characteristics in intraspecific hybrids of *Betula pubescens*. (a) PC1 represents a principal component that encompasses tree height, number of main ramets, and ramet angle and represents a gradient from *tortuosa*-like plants that are short, prostrate, and with many ramets to *pubescens*-like trees with opposite characteristics. (b) Relationships between leaf FA and tree height, which is the only variable in PC1 with a significant genetic component and is therefore the best hybrid index.

TABLE 3. Results of ANOVAs of leaf fluctuating asymmetry between a tree-line and lower elevation garden and among pure (*Betula pubescens*, *B. nana*) and hybrid (*B. nana* × *B. pubescens*) birch taxa.

Source of variation	df	MS	F	P
Garden	1	0.013	8.0	0.015
Taxa	2	0.006	3.5	0.066
Garden × taxa	2	0.002	1.1	0.381
Family (Garden × taxa)†	12	0.002	1.7	0.100
Error (within families)	36			
Contrast				
Hybrid vs. pure	1	0.008	4.8	0.048

† Error term for garden, taxa and garden × taxa and contrast.

environmental origin in our data set (ramet angle, $F = 1.33$, $P = 0.290$; number of ramets, $F = 1.2$, $P = 0.349$), we then reran the analysis on tree height, which should be a better index of true introgression. This relationship was not significant, and thus intermediates between the subspecies had leaves that were not different in the amount of asymmetry (Fig. 4b).

Elevation stress and asymmetry

As hypothesized, leaf FA was higher in the tree line than the lower elevation garden (Table 3, Fig. 5), and again, hybrids had higher leaf FA than did the pure parents, although the difference was not quite as large as in the first data set (Table 2). There was some indication that *B. nana* was less effected by elevation stress (20% increase) than *B. pubescens* (47%) and that the hybrid effect was greater in the upper garden, but this interaction was not significant.

Ramet-angle variance did not differ between gardens ($F = 0.06$, $df = 1, 12$, $P = 0.82$) and there was no interaction between garden and taxa ($F = 0.16$, $df = 1, 12$, $P = 0.85$). However, ramet variance was significantly higher in hybrid trees compared to the two

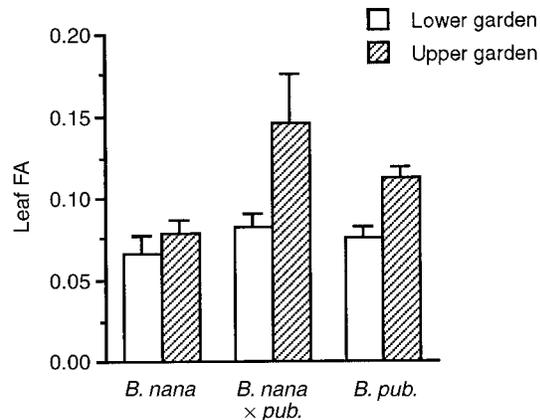


FIG. 5. Leaf fluctuating asymmetry (FA), adjusted for leaf size, in *Betula nana*, *B. pubescens*, and *B. nana* × *pubescens* hybrid trees from the tree-line and lower elevation common garden ($n = 9$).

pure taxa (taxa main effect, $F = 5.0$, $df = 2, 12$, $P < 0.03$; contrast, hybrid vs. pure, $F = 9.59$, $P < 0.01$).

DISCUSSION

Predictions of how fluctuating asymmetry (FA) will be affected by hybridization fall under two general hypotheses (reviewed in Palmer and Strobeck [1986] and Markow [1995]). The heterozygosity hypothesis predicts that hybrids will have reduced fluctuating asymmetry compared to their parents because of increased heterozygosity in hybrids (Soule 1979, Mitton 1993). The coadaptation hypothesis predicts that FA arises from the disruption of coadapted gene complexes, which occurs in response to hybridization, and as a result hybrids should have higher fluctuating asymmetry (Clarke 1993). These two hypotheses may not be mutually exclusive, but may operate at different levels, depending on how closely related the parents of the hybrids are (Markow 1995). For example, hybrids of closely related parents (e.g., intraspecific crosses) may have little or decreased asymmetry (from reduced inbreeding depression), whereas hybrids of distantly related parents (e.g., interspecific crosses) may have higher FA (Markow 1995). The length of time since the hybridization event is also potentially important, because the organism may re-evolve coadapted gene complexes (Felley 1980, Graham and Felley 1985).

Our study was the first (as far as we know) to test for the effects of interspecific hybridization and elevation stress on plant leaf asymmetry. We did this by sampling leaves from trees growing within common gardens. The major advantages of common gardens included (1) the origins of seeds were known, (2) trees were growing in natural soil, and (3) the effects of phenotypic plasticity in trees caused by differential shading from neighbors (e.g., Solangaarachi and Harper 1989), which may complicate interpretations of asymmetry (Palmer 1996), should have been minimized because plants were evenly spaced 1 m apart and 2 m from the next row, and therefore received little differential shading. By knowing the origin of seeds, we were able to partition the variance associated with the environment (i.e., upper vs. lower gardens) from genetic variance (i.e., between hybrid and pure taxa).

In all cases, we found that interspecific hybrids had higher FA than did parental taxa. Furthermore, in the intraspecific studies with *B. pubescens*, there was no relationship between tree height (intraspecific hybrid index) and leaf asymmetry. Thus, these data, and the data of Freeman et al. (1995) who found little difference in asymmetry of hybrids between two subspecies of *Artemisia tridentata*, supports the ideas of Markow (1995) that the changes in asymmetry observed from hybridization will be a function of how closely related the parental taxa are, with increases in asymmetry found from interspecific hybridization but limited effect in asymmetry from intraspecific hybridization. We

also found a smaller hybrid effect between *B. nana* and *B. pendula*, which both have 28 chromosomes, than we did in the other two crosses, which involved crossing parent taxa with 28 and 56 chromosomes. Thus, the similarity of chromosome numbers between taxa may also be of importance.

Within *B. pubescens*, individuals with environmentally derived *tortuosa*-like characteristics (i.e., shorter, more prostrate trees with many ramets) had higher leaf FA than did trees with more *pubescens*-like characteristics. Trees with *tortuosa* characteristics are also the most likely to encounter outbreaks from the autumnal moth (*Epirrita autumnata*), which in some years completely defoliates and sometimes kills trees in an area (Tenow 1972, Kallio et al. 1983, Haukioja et al. 1988). This suggests that leaf FA may be negatively correlated with some aspect of resistance of these plants to herbivory. However, Hanhimäki et al. (1994), who studied the same *B. nana* × *pubescens* hybrid trees, did not find significant differences in herbivore performance between hybrid and pure taxa, and this suggests that forage quality was not affected by hybridization in *Betula*.

There was no additive genetic variation in leaf or ramet FA (no family within taxa interaction), and this was in agreement with another analysis of nine families of birch from the upper garden (B. J. Wilsey and I. Saloniemi, unpublished data). Thus, since all of these taxa except *B. nana* are close to the northern/altitudinal limit of their range, it suggests that families do not vary in their ability to buffer themselves against the severity of the environment. As a result, there may be little or no variation in leaf developmental stability for natural selection to act upon if environments change and become more or less stressful.

Trees growing in the tree-line garden, which is at the very limit for the *B. pubescens* altitudinal distribution, had higher leaf FA but similar levels of ramet asymmetry than did trees growing in the lower garden, which is located in typical *B. pubescens tortuosa* habitat. *B. pubescens* trees growing in high-elevation sites probably encounter more severe conditions when compared to trees in the lower garden, including higher winds and lower spring and early summer temperatures. These factors, together with the reduced ability of birch to obtain the limiting nutrient nitrogen (Hinneri 1974) from cold soils (Karlsson and Nordell 1996) could have had a negative effect on current leaf growth and development. Kozlov et al. (1996) found that leaf asymmetry increased with pollution load in *B. pubescens* and *B. pendula*, and this further suggests that leaf asymmetry might be a general indicator of the severity of the environment in *Betula*, and perhaps other plant species (e.g., Freeman et al. 1993). Ramet asymmetry, which develops over several years early in the life of the tree, was not as sensitive to elevation stress. This suggests that leaf asymmetry may be a better indicator of stress than measures of ramets.

In conclusion, leaf FA increased in *Betula* as a result of hybridization and elevation stress. Since both of these factors are important in the tree-line ecology of *B. pubescens*, it suggests that this measure might be a useful indicator of environmental stress and distributional limits of this and possibly other plant species.

ACKNOWLEDGMENTS

We especially thank Anna Loan-Wilsey for help in field sampling and for making leaf asymmetry measurements. We also thank Sinikka Hanhimäki, Seppo Neuvonen, and staff at Kevo Subarctic Research Station for help in locating trees in the common gardens and Gray Stirling, Catherine Potvin, Leanne Jablonski, and two anonymous reviewers for commenting on an earlier version of this manuscript. The University of Turku and the Science Academy of Finland funded the research reported here.

LITERATURE CITED

- Anamthawat-Jonsson, K., and T. Tomasson. 1990. Cytogenetics of hybrid introgression in Icelandic birch. *Hereditas* **112**:65–70.
- Bagchi, S., and S. Iyama. 1983. Radiation induced developmental instability in *Arabidopsis thaliana*. *Theoretical and Applied Genetics* **65**:85–92.
- Barret, S. C. H., and L. D. Harder. 1992. Floral variation in *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae). II. Effects of development and environment on the formation of selfing flowers. *Journal of Evolutionary Biology* **5**:83–107.
- Boecklen, W. J., and R. Spellenberg. 1990. Structure of herbivore communities in two oak (*Quercus* sp.) hybrid zones. *Oecologia* **85**:92–100.
- Clarke, G. M. 1993. Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality. *Environmental Pollution* **82**:207–211.
- Elkington, T. T. 1968. Introgressive hybridization between *Betula nana* L. and *B. pubescens* Ehrh. in north-west Iceland. *New Phytologist* **67**:109–118.
- Eriksson, G., and A. Jonsson. 1986. A review of the genetics of *Betula*. *Scandinavian Journal of Forest Research* **1**:421–434.
- Felley, J. 1980. Analysis of morphology and asymmetry in bluegill sunfish (*Lepomis macrochirus*) in the southeastern United States. *Copeia* **80**:18–29.
- Floate, K. D., and T. G. Whitham. 1993. The “hybrid bridge” hypothesis: host shifting via plant hybrid swarms? *American Naturalist* **141**:651–662.
- Freeman, D. C., J. H. Graham, D. W. Byrd, E. Durant McArthur, and W. A. Turner. 1995. Narrow hybrid zone between two subspecies of big sagebrush, *Artemisia tridentata* (Asteraceae). III. Developmental instability. *American Journal of Botany* **82**:1144–1152.
- Freeman, D. C., J. H. Graham, and J. M. Emlen. 1993. Developmental stability in plants: symmetries, stress and epigenesis. *Genetica* **89**:97–119.
- Fritz, R. S., C. M. Nichols-Orians, and S. J. Brunfield. 1994. Interspecific hybridization of plants and resistance to herbivores: hypotheses, genetics, and variable responses in a diverse community. *Oecologia* **97**:106–117.
- Gange, A. C. 1995. Aphid performance in an alder (*Alnus*) hybrid zone. *Ecology* **76**:2074–2083.
- Graham, J. H., and J. D. Felley. 1985. Genomic coadaptation and developmental stability within introgressed populations of *Enneacanthus gloriosus* and *E. obesus* (Pisces, Centrarchidae). *Evolution* **39**:104–114.
- Grant, V. 1981. *Plant speciation*. Columbia University Press, New York, New York, USA.
- Hanhimäki, S., J. Senn, and E. Haukioja. 1994. Performance of insect herbivores on hybridizing trees: the case of the subarctic birches. *Journal of Animal Ecology* **63**:163–175.
- Haukioja, E., S. Neuvonen, S. Hanhimäki, and P. Niemel. 1988. The autumnal moth *Epirrita autumnata* in Fennoscandia. Pages 163–178 in A. A. Berryman, editor. *Dynamics of forest insect populations: patterns, causes, and management strategies*. Plenum, New York, New York, USA.
- Haukioja, E., K. Ruohomäki, J. Senn, J. Suomela, and M. Walls. 1990. Consequences of herbivory in the mountain birch (*Betula pubescens* ssp. *tortuosa*): importance of the functional organization of the tree. *Oecologia* **82**:238–247.
- Hinneri, S. 1974. Podzolic processes and bioelement pools in subarctic forest soils at the Kevo station, Finnish Lapland. Report from Kevo Subarctic Research Station **11**:26–34.
- Kallio, P., and Y. Mäkinen. 1978. Vascular flora of Inari, Lapland. 4. Betulaceae. Report from Kevo Subarctic Research Station **14**:38–63.
- Kallio, P., S. Niemi, and M. Sulkinoja. 1983. The fennoscandian birch and its evolution in the marginal forest zone. Pages 101–111 in P. Morisset and S. Payette, editors. *Tree-line ecology. Proceedings of the Northern Quebec Tree-Line Conference. Collection Nordicana*. Laval University, Quebec, Canada.
- Karlsson, P. S., and K. O. Nordell. 1996. Effects of soil temperature on the nitrogen economy and growth of mountain birch seedlings near its presumed lower temperature distribution limit. *Ecoscience* **3**:183–189.
- Kozlov, M., B. J. Wilsey, J. Koricheva, and E. Haukioja. 1996. Fluctuating asymmetry of birch leaves increases under pollution impact. *Journal of Applied Ecology* **33**:1489–1495.
- Kozłowski, T. T., and J. J. Clausen. 1966. Shoot growth characteristics of heterophyllous woody plants. *Canadian Journal of Botany* **44**:827–843.
- Kryazheva, N. G., E. K. Chistyakova, and V. M. Zakharov. 1996. Analysis of development stability of *Betula pendula* under conditions of chemical pollution. *Russian Journal of Ecology* **27**:422–424.
- Leary, R. F., and F. W. Allendorf. 1989. Fluctuating asymmetry as an indicator or stress: implications for conservation biology. *Trends in Ecology and Evolution* **4**:214–217.
- Levin, D. A. 1970. Developmental instability in species and hybrids of *Liatrus*. *Evolution* **24**:613–624.
- Markow, T. A. 1995. Evolutionary ecology and developmental instability. *Annual Review of Entomology* **40**:105–120.
- McLellan, T. 1990. Development of differences in leaf shape in *Begonia dregei* (Begoniaceae). *American Journal of Botany* **77**:323–337.
- . 1993. The roles of heterochrony and heteroblasty in the diversification of leaf shapes in *Begonia dregei* (Begoniaceae). *American Journal of Botany* **80**:796–804.
- Mather, K. 1953. Genetical control of stability in development. *Heredity* **7**:297–336.
- Møller, A. P. 1995. Leaf-mining insects and fluctuating asymmetry in elm *Ulmus glabra* leaves. *Journal of Animal Ecology* **64**:697–707.
- Moorehead, J. R., M. L. Taper, and T. J. Case. 1993. Utilization of hybrid oak hosts by a monophagous gall wasp: how little host character is sufficient? *Oecologia* **95**:385–392.
- Palmer, A. R. 1996. Waltzing with asymmetry. *BioScience* **46**:518–532.
- Palmer, A. R., and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology and Systematics* **17**:391–421.
- Parsons, P. A. 1991. Fluctuating asymmetry: a biological

- monitor of environmental and genomic stress. *Heredity* **68**:361–364.
- Paxman, G. J. 1956. Differentiation and stability in the development of *Nicotiana rustica*. *Annals of Botany* **20**:331–347.
- SAS Institute. 1988. SAS procedures guide. Release 6.03. SAS Institute, Cary, North Carolina, USA.
- Sherry, R. A., and E. M. Lord. 1996. Developmental stability in leaves of *Clarkia tembloriensis* (Onagraceae) as related to population outcrossing rates and heterozygosity. *Evolution* **50**:80–91.
- Solangaarachi, S. M., and J. L. Harper. 1989. The growth and asymmetry of neighbouring plants of white clover (*Trifolium repens* L.) *Oecologia* **78**:208–213.
- Soulé, M. E. 1979. Heterozygosity and developmental stability: another look. *Evolution* **33**:396–401.
- Tenow, O. 1972. The outbreaks of *Oporinia autumnata* Bkh. and *Operophtera* spp. (Lep., Geometridae) in the Scandinavian mountain chain and northern Finland 1862–1968. *Zoologiska Bidrag (Uppsala)*. Supplement **2**:1–107.
- Van Valen, L. 1962. A study of fluctuating asymmetry. *Evolution* **16**:125–142.
- Whitham, T. G. 1989. Plant hybrid zones as sinks for pests. *Science* **244**:1490–1493.