
Developmental Instability in Fragmented Populations of Prairie Phlox: A Cautionary Tale

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Abstract: Considerable attention has recently been focused on using levels of developmental instability among members of a population to detect environmental or genetic stresses on animals or plants. It is not yet clear, however, that high developmental instability in a sample of individuals always indicates environmental stress or poor genetic quality. We studied 13 fragmented populations of prairie phlox (*Phlox pilosa* L.) to test the hypothesis that developmental instability should decrease with increasing population size—as expected if small populations suffer genetic problems associated with inbreeding or are exposed to more environmental stress than larger populations. We used two different measures of developmental instability, each calculated for two different traits: radial asymmetry of flowers (for petal width and petal length) and modular fluctuating asymmetry of leaves (for leaf widths at two points along the leaf). There were weak but significant correlations among individuals for four of six pairwise combinations of these measures. Surprisingly, three of our four measures of developmental instability showed strong population size effects that were opposite to those expected: developmental instability increased with population size. We conclude that measures of developmental instability cannot be applied uncritically for biomonitoring without considerable knowledge of developmental mechanisms, natural history, and population biology of the species in question.

Inestabilidad en el Desarrollo de Poblaciones Fragmentadas de *Phlox pilosa*: Una Historia de Advertencia

Resumen: Recientemente se ha enfocado una considerable cantidad de atención hacia el uso de niveles de inestabilidad de desarrollo entre miembros de una población para detectar estrés ambiental o genético en animales o plantas. Sin embargo, aún no es claro a qué niveles de inestabilidad alta una muestra de individuos indica estrés ambiental o calidad genética pobre. Estudiamos 13 poblaciones fragmentadas de *Phlox pilosa* L. para probar la hipótesis de que la inestabilidad del desarrollo debe disminuir con un incremento en el tamaño poblacional (tal como se espera si poblaciones pequeñas sufren problemas genéticos asociados con intracruza o están expuestos a más estrés ambiental que las poblaciones grandes). Utilizamos dos medidas diferentes de inestabilidad del desarrollo, cada una calculada para dos características diferentes: asimetría radial de flores (para anchura y longitud del pétalo) y fluctuación modular de la asimetría de hojas (para anchura de la hoja en dos puntos a lo largo de la hoja). Existen correlaciones débiles pero significativas entre individuos de cuatro de las seis combinaciones pareadas de estas mediciones. Sorpresivamente, tres de las cuatro medidas de inestabilidad del desarrollo mostraron efectos fuertes del tamaño poblacional que fueron contrarios a lo esperado: la inestabilidad del desarrollo incrementó con el tamaño poblacional. Concluimos que las medidas de inestabilidad del desarrollo no pueden ser aplicadas para el bio-monitoreo sin un conocimiento considerable de los mecanismos de desarrollo, la historia natural y la biología poblacional de la especie en cuestión.

Introduction

Habitat fragmentation is one of the most pervasive and important effects of human activity on natural ecosys-

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Paper submitted February 26, 1998; revised manuscript accepted August 20, 1998.

tems (Saunders et al. 1991). Its consequences for animal and plant populations can include increased risk of extinction from stochastic fluctuations in population size (Lande 1988), changes in interactions with predators, prey, competitors, or mutualists (Krueess & Tscharnkte 1994; Groom 1998), and changes in genetic structure (inbreeding and loss of genetic variation; Barrett & Kohn 1991; Ellstrand & Elam 1993). In general, for these and other reasons the fitness of individuals in small populations is likely to be reduced (e.g., Lamont et al. 1993; Widén 1993; Aizen & Feinsinger 1994; Ågren 1996). (By fitness we mean realized reproductive success, and reduced fitness may reflect both genetic quality and environmental impacts.) But not all species suffer in fragmented habitats or small populations (Alexandersson & Ågren 1996; Molano-Flores 1997). To set conservation priorities we must be able to assess the relative threats posed by fragmentation to different species. Conservation biologists need rapid and reliable techniques for detecting reductions in fitness inflicted by fragmentation and other demographic and environmental insults.

Recently, considerable attention has been paid to developmental instability (DI), or the vulnerability of organisms to developmental perturbations, as an indicator of individual fitness and the health of populations (Palmer & Strobeck 1986; Parsons 1990; Freeman et al. 1993; Markow 1995). Measuring DI may be quicker and cheaper and may provide earlier stress detection than other monitoring methods. An individual shows DI when its development is disrupted by environmental stresses such as pollution, parasite loads, or temperature extremes, resulting in a phenotype perturbed from a presumed ideal. The extent to which a given stress disrupts development will depend on the stressed individual's overall genetic quality, which may be compromised by problems such as inbreeding, hybridization, or chromosomal abnormalities (Grant & Grant 1995; Palmer 1996; Sherry & Lord 1996a, 1996b). Therefore, measures of DI are expected to be greatest in populations that are environmentally stressed, genetically susceptible, or both. Such patterns are well known in animals (Palmer & Strobeck 1986; Markow 1995), although they are not universal and a few counterexamples exist (of higher DI in individuals of higher fitness) (Packer & Pusey 1993; Swaddle & Witter 1994). Much less is known about DI in plants, but associations of DI with genetic and environmental stresses have been reported (e.g., Freeman et al. 1993; Sherry & Lord 1996a, 1996b; Zvereva et al. 1997; Wilsey et al. 1998).

Habitat fragmentation is often associated with deterioration in environmental conditions, and small populations resulting from habitat fragmentation may be vulnerable to inbreeding and other genetic problems (Menges 1991; Ellstrand & Elam 1993; Heschel & Paige 1995). Developmental instability could therefore be a useful tool for assessing the consequences of fragmentation for

plant and animal populations. We are aware of only two studies relating DI directly to population size: Soulé (1967) for lizards and Schwaner (1990) for snakes; both found increased DI in small populations. We studied DI in prairie phlox (*Phlox pilosa* L. [Polemoniaceae]), a plant whose modern distribution reflects severe habitat fragmentation and whose many remnant populations are extremely small.

P. pilosa is an obligately outcrossing perennial plant of tallgrass prairies, which have undergone extraordinary fragmentation in North America since European settlement (Samson & Knopf 1994). Like many other prairie plants, *P. pilosa* is now largely limited to small prairie remnants along roadsides and railroad rights-of-way and in small preserves. *Phlox* plants in small populations are often exposed to environmental insults such as drift of herbicides from fields and rock dust from roads. Small populations show several indications of reduced fitness, including lower fruit and seed set, less movement of outcross pollen, and lower mycorrhizal infection rates (Kyhl 1997; Page 1997).

We asked whether patterns in developmental instability revealed effects of fragmentation on *P. pilosa* populations. We explored two related points: (1) whether two measures of DI (modular fluctuating asymmetry of leaves and radial asymmetry of flowers) are inversely related to *Phlox* population size and (2) whether these different measures are correlated across *Phlox* individuals and populations. We would expect inverse relationships with population size and positive correlations among measures if DI is a useful indicator of genetic and environmental stresses in fragmented plant populations.

Methods

Study Populations and Measurement of Developmental Instability

We estimated developmental instability of *Phlox pilosa* from 13 populations from prairie fragments in east-central Iowa (U.S.A.). These populations spanned more than three orders of magnitude in size and included a pioneer cemetery, roadsides, railroad rights-of-way, and prairie preserves (Table 1). We expressed population size as number of flowering stems (at peak flowering) because we could not reliably distinguish between ramets belonging to different genets. Nonflowering ramets were not counted because they are extremely difficult to locate in dense prairie vegetation. In any year, a genet might produce multiple flowering stems or might not flower at all, but flowering stems provide a proxy for the number of genetic individuals and allow us to order populations from small to large. Population sizes were determined by direct counts in small populations (<1000 stems) and by quadrat methods in large populations. Full site de-

Table 1. Characteristics, sizes, and sampling intensities for *Phlox pilosa* populations studied.

Population	Remnant type	Population size	Plants sampled for flowers	Plants sampled for leaves
Solon Prairie	preserve	3000 ^a	18	34
Williams Prairie	preserve	152 ^a	—	20
Rochester Cemetery	pioneer cemetery	29,000 ^a	18	31
Heinz Road	active railway	139 ^a	—	19
Hoover Trail	abandoned railway	68 ^a	—	19
Morse Trail N	abandoned railway	18 ^b	—	9
Morse Trail S	abandoned railway	83 ^b	—	12
Highway 965	roadside	112 ^a	—	20
Sutliff Road	roadside	1030 ^b	20	40
Upper Boulder Road E	roadside	1600 ^b	20	40
Upper Boulder Road W	roadside	8900 ^c	19	41
Red Bridge Road E	roadside	859 ^b	19	34
Red Bridge Road W	roadside	2100 ^b	12	19

^aHarmonic mean of population sizes, 1992–1996.^bHarmonic mean of population sizes, 1993–1996.^cHarmonic mean of population sizes, 1995–1996.

scriptions and details of population size estimation are provided by Kyhl (1997). Collections were made in the summer of 1996, but population sizes were based on counts made yearly between 1992 and 1996 (Table 1).

Developmental instability is reflected in deviations of the morphology of individuals away from underlying developmental targets. The most common measure of DI, fluctuating asymmetry, exploits the fact that for bilaterally symmetric structures the target can be assumed to be perfect bilateral symmetry (Palmer 1996). Developmental instability can also be assessed for other developmental targets, as long as the target is known or can be estimated by comparing multiple measurements on a single genetic individual (Freeman et al. 1993). Because of their modular construction, plants are ideal subjects for the latter strategy. The DI of plants has been assessed via deviations from translational and rotational symmetry, variation in petal or leaflet number among flowers or leaves, and even variation in terpene profiles among branches (Freeman et al. 1993). We used two different measures of developmental instability. The first, radial asymmetry of flowers, assesses deviations from perfect radial symmetry in petal lengths and widths. The second, modular fluctuating asymmetry of leaves, exploits the modular construction of plants to compare asymmetry of *Phlox* leaves to an estimable developmental target. *Phlox* leaves are directionally asymmetric, so we assessed variance among leaves in how closely they approached the presumed target asymmetry (estimated by the average asymmetry within a plant). In both cases we were interested in asymmetry differences among populations and did not attempt to interpret absolute levels of asymmetry.

Flower Radial Asymmetry

We collected flowers from 12–20 haphazardly chosen stems in our seven largest populations (Table 1). We did

not collect flowers from smaller populations because doing so might have substantially reduced seed sets in those populations. We collected 1–15 (mean 5.6) flowers per stem, making repeat visits to collect recently opened, undamaged flowers. Flowers were preserved in 70% ethanol.

To hold each flower open and flat for measurement, we inserted its corolla tube into a well drilled in a plexiglass plate and placed a glass microscope slide over the petals (corolla lobes). For each of the five petals, we measured width at the widest point and length from the center of the corolla tube (Fig. 1a) at 6.3× magnification under a dissecting microscope. For consistency, one of us (M.A.C.) made all flower measurements.

We measured deviations from radial symmetry in petal width and length (a flower shows radial asymmetry if some petals are wider or longer than others). Radial asymmetry (RA) has been used as an index of developmental instability for flowers (e.g., Møller & Eriksson 1994; Sherry & Lord 1996a) and for palmately compound and veined leaves (Freeman et al. 1993). We calculated measures of RA for plants and for populations. Our raw asymmetry values for an individual flower were the variances of the petal width and length measurements. We did not correct for flower size because the asymmetry of a flower did not depend on its size. For a plant our RA measures were the means of the flower-level variances. For a population, our RA measures were the means of the plant-level RAs.

Leaf Modular Fluctuating Asymmetry

We collected leaves from both flowering *Phlox* stems (using the same stems from which we collected flowers) and vegetative *Phlox* stems in all 13 of our populations. We haphazardly marked between 9 and 41 plants in each population, with fewer than 19 plants only in the two smallest populations (Table 1). At the end of the

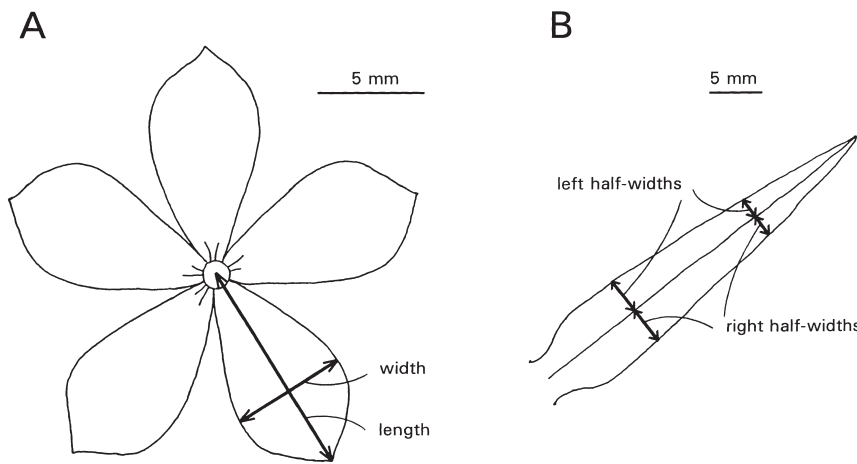


Figure 1. *Phlox pilosa* structures measured for estimates of developmental instability: typical flower showing measurements for calculation of radial asymmetry (a) and typical leaf (abaxial surface) showing measurements for calculation of modular fluctuating asymmetry (b).

flowering period, we collected all undamaged leaves from each plant (3–24, mean = 7.1 leaves/plant). Leaves were preserved in 70% ethanol.

Before measuring leaves, we soaked them in water to restore flexibility. Each leaf was held flat and measured at 16 \times magnification under a dissecting microscope. We measured left and right half-widths (perpendicular distances from the midrib to the left and right margins of the leaf) at one-third and two-thirds of the leaf's length (Fig. 1b). For consistency, one of us (M.L.B.) made all leaf measurements. We treated the two sets of half-widths separately, as potentially independent characters.

Our measures of raw asymmetry (not modular fluctuating asymmetry) for a single leaf were the signed differences between the left and right half-widths ($L - R$) at each measurement point. We did not correct for leaf size because the asymmetry of a leaf did not depend on its size. We tested for directional asymmetry and antisymmetry by examining the mean and kurtosis of the half-width differences. After detecting significant directional asymmetry (but not antisymmetry), we calculated the average directional asymmetry per plant (the mean of the half-width differences for that plant) and used one-way analysis of variance to compare directional asymmetry among populations. We checked for within-plant position effects on leaf asymmetry with a separate collection of leaves (in 1998; all leaves from 10 plants from the Solon Prairie population) for which we recorded the position of each leaf along the stem. We tested for the effects of position on ($L - R$) using both linear regressions and analyses of variance (treating position as continuous and discrete, respectively).

Our leaf measurements resemble those commonly taken for the calculation of fluctuating asymmetry (Palmer & Strobeck 1986). Because *Phlox* leaves are directionally asymmetric, however, we cannot interpret our raw leaf asymmetries as indicators of developmental instability (Palmer & Strobeck 1992). Instead, we measured multi-

ple leaves per stem to assess, for each plant, *variability* in asymmetry among leaves. The magnitude of morphological variability among modules, when those modules are genetically identical, increases with DI (Freeman et al. 1993; Sherry & Lord 1996a). We calculated the variance of half-width differences ($L - R$) among leaves within a plant. In a plant showing perfect developmental stability, all leaves would be identically asymmetric and the variance of ($L - R$) would be zero. For plants showing more DI, among-leaf variance in ($L - R$) would be larger. We refer to within-plant variance in ($L - R$) as modular fluctuating asymmetry (MFA) to emphasize that, although it (like fluctuating asymmetry) is based on bilateral asymmetry and reflects DI, it is measured over a set of modular structures to remove genetically determined directional asymmetry (Palmer & Strobeck 1992).

As we did for flowers, we considered MFA both for individual plants and for populations. Our MFA measures for a population were the means of the plant-level MFA measures. We refer to MFA measures (at the plant or population level) for the one-third-length and two-thirds-length points as leaf MFA1 and leaf MFA2.

Estimating Measurement Error

Because variation introduced by measurement error is expected to resemble variation arising from developmental instability, we estimated measurement error by measuring subsamples of our flower and leaf collections twice. As in most studies (Palmer & Strobeck 1986), measurement error was substantial compared to true asymmetry at the leaf or flower level (often 10–40% of true asymmetry). Measurement error, however, was not correlated with true asymmetry and did not vary over the course of our measurements. Measurement error may therefore have inflated our absolute MFA and RA measurements, but it did not produce or exaggerate patterns among characters, plants, or populations. In fact, it makes our estimates of the strength of such patterns

conservative. Because we were interested only in patterns and not in absolute levels of MFA or RA, we ignored the contribution of measurement error in all subsequent analyses.

Statistical Analysis

We used analysis of covariance to test for differences in leaf MFA between flowering and vegetative stems at the population level (the covariate was population size). Because there was no significant effect of flowering status on leaf MFA (both $F_{1,23} < 3.0$, $p > 0.09$), we ignored the distinction between flowering and vegetative stems in further analyses.

We calculated Pearson's correlation coefficients to assess relationships in asymmetry (MFA or RA) among characters at the plant and population levels. We used linear regression to test for dependence of population-level MFA and RA measures on log-transformed population sizes. Unweighted regressions and those weighting values for each population by their sample sizes (Palmer & Strobeck 1986) gave similar results; we report only the unweighted regressions. All statistical analyses were conducted with SAS version 6.04 (SAS Institute 1988).

Results

Both flower and leaf sizes (petal and leaf lengths and widths) varied significantly but only slightly ($0.07 < r^2 < 0.31$, data not shown) among populations. In no case was asymmetry related to the size of a structure (for flowers, correlations of petal width and length variances with average widths and lengths, both $r < 0.05$, $p > 0.27$, $n = 681$; for leaves, correlations of unsigned half-width differences with leaf lengths, both $r < 0.03$, $p > 0.18$, $n = 2384$). There were no position effects on leaf asymmetries (all $r^2 < 0.05$, $p > 0.14$).

Leaves showed slight but significant directional asymmetry at both measurement points (left sides on average about 4% wider than right; both $t > 18$, $p < 0.0001$, $n = 2384$). Directional asymmetry did not differ among populations (both $F_{12,324} < 1.65$, $p > 0.075$, $r^2 < 0.06$). There was no antisymmetry: both raw leaf asymmetries

were unimodal and significantly leptokurtic (both $g_2 > 2.0$).

Correlations between asymmetries in different characters were generally weak, but 5 of 12 were significant (Table 2). All 5 correlations remained significant after sequential Bonferroni adjustment over the six individual-level tests and the six population-level tests. Correlations between the two leaf characters or between the two petal characters tended to be stronger than correlations between petal characters and leaf characters. The strongest correlations were those measured at the population level between leaf MFA1 and leaf MFA2 and between petal width RA and petal length RA (although the latter had a small sample size and was not significant).

Three of the four characters we studied (leaf MFA1, leaf MFA2, and petal length RA) showed significant and positive relationships between population-level asymmetry and population size (Fig. 2a-c). The relationship between petal width RA and population size was also positive but was not significant (Fig. 2d). For the three significant relationships, the fractions of variance in MFA or RA explained by population size were large (46–70%), and the relationships were robust (regressions remain significant upon deletion of any single population).

Discussion

Weak Correlations in Asymmetry among Characters

If asymmetries in particular characters are related to overall individual quality or to the levels of stress experienced by individuals during development, then asymmetries in different characters ought to be correlated across individuals. Most studies, however, have failed to detect such correlations (Palmer & Strobeck 1986; Sherry & Lord 1996a, 1996b), and theoretical models suggest that they should be small (Leung & Forbes 1997). The weak ($r \approx 0.3$) but significant correlations for four of six pairwise combinations of characters (Table 2, above diagonal) suggest that large sample sizes will be needed to detect these relationships (see also Leamy 1993). The correlation was even weak ($r = 0.30$) for leaf MFA1 and leaf MFA2, similar measurements on the same structures. Other studies have found weak but significant

Table 2. Correlations in asymmetries among traits^a calculated at the plant (above diagonal) and population (below diagonal) levels.

	Leaf MFA1			Leaf MFA2			Petal width RA			Petal length RA		
	r	n	p ^b	r	n	p ^b	r	n	p	r	n	p ^b
Leaf MFA1	—	—	—	0.30	336	0.0001*	0.01	110	0.93	0.26	110	0.007*
Leaf MFA2	0.92	13	0.0001*	—	—	—	0.07	110	0.48	0.32	110	0.0006*
Petal width RA	0.05	7	0.92	0.11	7	0.81	—	—	—	0.26	126	0.004*
Petal length RA	0.12	7	0.79	−0.06	7	0.90	0.49	7	0.27	—	—	—

^aMFA, modular fluctuating asymmetry; RA, radial asymmetry.

^bValues marked with an asterisk are correlations significant ($p < 0.05$) after sequential Bonferroni adjustment.

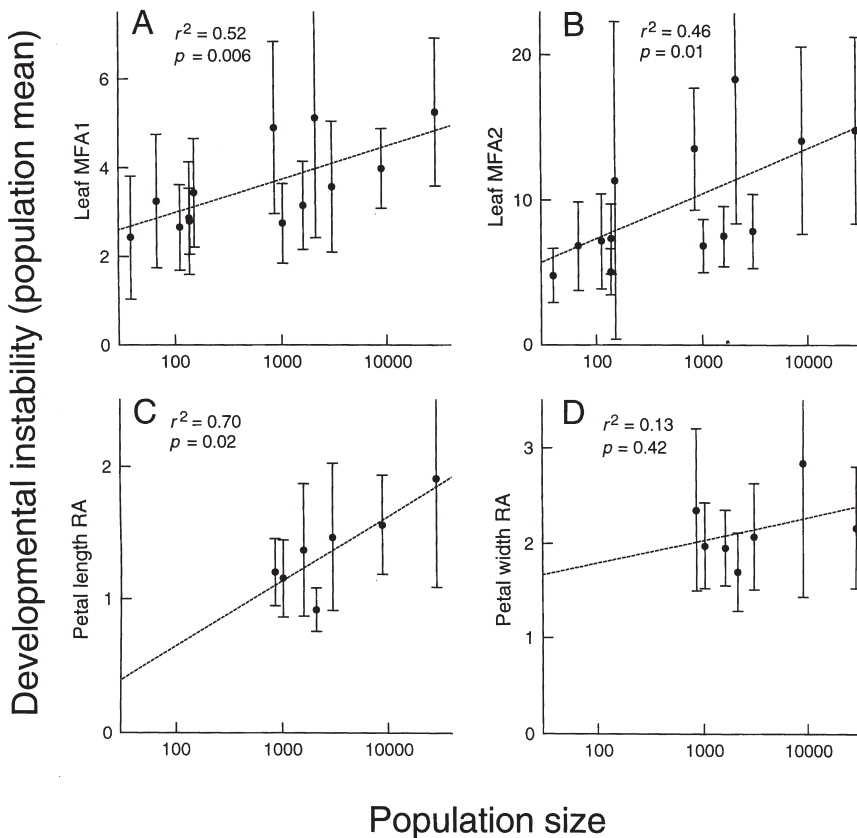


Figure 2. Patterns in developmental instability with population size: modular fluctuating asymmetry of leaves measured at one-third of leaf length (a); modular fluctuating asymmetry of leaves measured at two-thirds of leaf length (b); radial asymmetry in petal length (c); and radial asymmetry in petal width (d). Regression lines, r^2 values, and p values are from least-squares regression analyses of the population-level instability measures. Error bars show ± 2 SE.

concordance among asymmetries for different characters (Dufour & Weatherhead 1996; Kozlov et al. 1996).

Correlations among asymmetries in different characters are detected more often at the population level (Palmer & Strobeck 1986; Leamy 1993), but our data are mixed: a strong correlation between leaf characters and a strong (but not significant) correlation between petal characters, but no sign of correlations between petal and leaf characters (Table 2, below diagonal; see also Sakai & Shimamoto 1965). Clearly, patterns in DI can be interpreted with confidence only when they are determined for multiple characters (see also Zhivotovsky 1992).

Increasing Developmental Instability with Population Size

For three of four characters (leaf MFA1 and MFA2, and petal length RA), we found significant positive relationships between population size and DI, and for the fourth character (petal width RA) the trend was in the same direction (Fig. 2). Our conclusions are particularly robust (Palmer 1994) because measures of DI based on the four characters were only weakly correlated across individuals or populations. Furthermore, leaf MFA and flower RA were in agreement despite being based on different plant structures and referring to different types of symmetry. Our results are directly opposite those of two previous studies of the relationship between DI and

population size: Schwaner (1990, our reanalysis of data in his Table 1) found significantly more scale anomalies in snakes from smaller populations, and Soulé (1967) found significantly higher fluctuating asymmetry in lizard populations from small islands.

The positive relationships between *Phlox* population size and developmental instability were entirely unexpected and leave us with only two possibilities: either *Phlox* plants in small prairie fragments actually have higher fitness than those in large fragments (whether this reflects higher genetic quality or milder environmental conditions), or plants in small populations are disadvantaged but our measures of DI fail to reflect this fact.

We do not believe that *Phlox* plants in small populations have higher fitness. These plants have lower fruit and seed set in some years because of decreased amounts and quality of pollen arriving to stigmas (Kyhl 1997). Poor pollination can lead to inbreeding and to reduced competition among pollen tubes and ovules (Niesenbaum & Casper 1994), and the ultimate result is likely to be reductions in the genetic quality of progeny. Environmental stresses are obvious in small populations as well: *Phlox* plants in small populations (typically located along roadsides or railroads) are exposed to runoff and drift of herbicides, pesticides, rock dust, and salt from adjacent fields and roads (S.B.H. & S.D.H., personal observations) and often experience reduced mycor-

rhizal infection rates (Page 1997). A relationship between inverse fitness and population size is not impossible in general, but none of the potential mechanisms seems likely for *P. pilosa*. Fitness may decline with population size among plants using deceptive pollination (Alexandersson & Ågren 1996), but *Phlox* rewards its pollinators. Similar declines may arise when large populations support a higher diversity or density of insect herbivores (Strong et al. 1984), but herbivore damage to *Phlox* is uniformly rare (S.B.H. & S.D.H., personal observations). If large populations are more crowded, plants might suffer more competition, but our small *Phlox* populations are small because their extent is limited, not because *Phlox* density (or total plant density) is low. Finally, outbreeding in large populations might lower fitness through disruption of coadapted gene complexes, but this is most likely when those large populations result from the recent contact of locally adapted forms (Ross & Robertson 1990; Graham 1992; Wilsey et al. 1998). For *Phlox*, even our largest populations are tiny remnants of a much larger and continuous presettlement distribution.

Two hypotheses consistent with our results allow for higher environmental or genetic stress in small populations but are variations on the theme that plants in large populations might be victims of their own success (for a similar argument see Swaddle and Witter 1994). First, higher developmental instability in large populations could result from the physiological stress of sexual reproduction, which diverts resources to maturing fruits and seeds, perhaps impoverishing somatic tissue that develops later; large populations do have (in some years) higher fruit and seed sets. The effects of reproduction in one year, however, would have to carry over to affect development in the next because we collected flowers and leaves that developed before any fruits were set. This hypothesis also assumes no offsetting effect of allocation to asexual reproduction. Second, lower DI in small populations might result because intense selection has purged those populations of inferior genotypes that persist in larger, less stressed populations. Both of these hypotheses require a further assumption: that the increase in DI caused directly by stresses in small populations is outweighed by the decrease in DI resulting because small populations refrain from sexual reproduction and/or have been purged by selection. We are aware of neither theory nor data pertaining to this assumption, although Polak (1997) has proposed a model along similar lines to account for high fluctuating asymmetry in parasite-resistant individuals. In any case, the kind of elevated stress envisioned for large populations under these hypotheses is evidence of their success, not of their need for conservation.

Because an inverse relationship between population size and fitness seems unlikely for *Phlox*, we are left with the conclusion that measures of developmental in-

stability are probably not reliable indicators of the impacts of fragmentation on *Phlox* populations. Therefore, we urge that measures of DI not be applied uncritically or without some understanding of the nature of the suspected stressors and their effects on fitness (and perhaps better understanding of developmental mechanisms; Graham et al. 1993). Although others have advised caution in the study of DI, often on methodological grounds (Palmer & Strobeck 1992; Markow 1995; Palmer 1996), the overall reaction to DI as a tool for studies of conservation biology, population genetics, and sexual selection has been enthusiastic (Palmer 1996). Many studies fail to find predicted patterns in DI (Palmer & Strobeck 1986), but our study lines up instead with a handful of others (Packer & Pusey 1993; Swaddle & Witter 1994) reporting a significant pattern in a direction opposite to that expected. Had we interpreted patterns in DI uncritically, we would have identified precisely the wrong populations as needing conservation attention. Recognizing this mistake is not difficult with our *Phlox* system, but where the potential stresses are more subtle it could be much more problematic. Researchers should be aware that measures of developmental instability cannot be confidently interpreted without a broader understanding of the natural history and population biology of the organisms in question.

Acknowledgments

We thank P. Aldrich, D. Ayres, M. Cattell, D. Cipollini, L. González-Guzmán, S. Juliano, B. Robinson, and B. Wilsey for discussion, and R. Cruden, E. Main, J. Graham, K. Terpstra, and two anonymous reviewers for comments on the manuscript. J. Dyer and R. Sines helped in field and lab, and K. Terpstra drew Fig. 1. This research was supported in part by funds for S.B.H. from the University of Iowa.

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