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Effects of inbreeding on phenotypic plasticity in cultivated *Phlox*

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Summary. Inbreeding is known to increase developmental instability in outbreeding plants, and it has been argued that phenotypic plasticity in response to environmental variation might be similarly affected. To investigate whether phenotypic plasticity is altered by inbreeding, an outcrossed group and three successive generations of inbred cultivated *Phlox drummondii* were grown in six different treatments (Control, Low Water, Low Nutrient, Early and Late Leaf Removal, and Small Pots). Twelve plant characters were measured to determine the effects of the treatments and inbreeding. For those characters where inbreeding level by treatment interaction was indicated, the amounts and patterns of plasticity were examined to determine the source of the interaction. Despite substantial evidence for inbreeding depression of plant vigor and fecundity, there was no indication of an increase in the amount of phenotypic plasticity with progressive inbreeding. There was also no evidence that inbreeding systematically disrupts the pattern of plastic response to the environment.

Key words: Amount of phenotypic plasticity – Pattern of phenotypic plasticity – Genotype × environment interaction – Inbreeding depression – *Phlox drummondii*

Introduction

There is considerable evidence that inbreeding increases asymmetry of paired structures, frequency of extra or missing parts, and other forms of developmental instability in predominantly outbreeding animals and plants (Lerner 1954; Rendel 1967; Levin 1970; Soule 1982). Such instability results from the expression of homozygous deleterious recessive genes, and the increased sensitivity to 'internal' environmental variation due to homozygosity (Soule 1982). If inbreeding causes a decline in developmental canalization, it follows that inbreeding also may lead to increased developmental (phenotypic) plasticity in response to external environmental variation (Bradshaw 1965). Whether this line of reasoning is valid remains to be determined, because studies of inbreeding depression typically have not considered this issue.

The purpose of this study was to determine the extent to which progressive inbreeding leads to alterations in phenotypic plasticity, and how such alterations are related to inbreeding depression (expressed as plant size and fecundity) in *Phlox drummondii*. This species is almost exclusively outbreeding, so in order to facilitate inbreeding, the study was conducted on a self-compatible cultivar whose genetic system still favors outcrossing (Levin 1975).

Species, experiment and analysis

Wild *Phlox drummondii* is a self-incompatible winter annual native to central Texas. Its cultivars are typically self-compatible, allowing easy inbreeding, and have been selected for floral characteristics as well as for branching and stature. The cultivar used in this study, 'Dwarf Crimson Beauty', was obtained from Sluis and Groot, Enkhuizen, Netherlands.

Four levels of inbreeding were compared using seven families of 'Dwarf Crimson Beauty': 1) an outcrossed (0) group derived from hand pollinations between plants grown from a bulk seed source, 2) one generation inbred (I) plants

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derived from selfed seed from the same plants used to generate outcrossed seed, 3) second generation inbreds (II) derived from selfing in inbred I plants, and 4) third generation inbreds (III) from selfed seed of inbred II plants. Theoretically, each generation of inbreeding reduces the average heterozygosity by one half.

Plants were grown singly in sand-filled pots in the greenhouse in one of 6 treatments: plants in treatments 1–5 were grown in 4 inch pots; 1) Control – daily watering to keep the sand moist, and biweekly fertilization with 20:20:20 NPK and micronutrients; 2) Low water – water every other day, fertilization as in the Control; 3) Low nutrients – no nutrients after week 5, with daily watering; 4) Early leaf removal – 50% of the plant's expanded leaves were removed at week 4, water and nutrients as in the Control; 5) Late leaf removal – 50% of the plant's expanded leaves were removed at week 10, water and nutrients as in the Control; 6) Small pots – plants were grown in 2 inch pots which hold only one-third as much sand as 4 inch pots. Plants in these pots had restricted space for root growth and also were subject to occasional wilting despite daily watering; nutrients as in the Control.

The 6 treatments were replicated in 3 randomized blocks. Fifteen plants of inbreeding level 0 and 20 plants each of levels I, II and III were grown in each treatment. All families and inbreeding levels were represented and were randomized within each treatment block. A total of 450 plants were grown, 75 per treatment.

Plants were harvested after 21 weeks and dried and the following characters were measured: 1) Shoot dry weight (g); 2) Root dry weight (g); 3) Root/shoot ratio; 4) Plant height (cm); 5) Total branches (on main axis); 6) Ratio of basal (below 7.5 cm on main axis) to total branches; 7) Average internode length (main axis height/total branches); 8) Days to flower; 9) Total flower production; 10) Flowers/gram (total flowers/total weight); 11) Capsule production; 12) % Selfing (no. of capsules/total flowers).

Data analysis

Data were analyzed by means of analysis of variance (ANOVA) using the General Linear Models procedure of SAS (1982). Each of the 12 characters was analyzed with the model: Character = Treatment Inbreeding level Family Block Treatment × Inbreeding level. Each variable was tested for significance using the F-statistic derived from the Type III sums of squares. Type III sums of squares are computations of the variance due to the independent variable after all other variables are accounted for (SAS 1982). Family and block effects were included in the model to control these sources of variation and, although occasionally significant, these effects are not of interest in the context of this study and will not be examined further. There were no significant family × treatment interactions allowing the use of the inbreeding level as a homogeneous unit.

A significant interaction term in the full model indicates only that the plastic responses of one or more of the inbreeding levels differs from the average response of all levels. Because the differences in plasticity between individual inbreeding levels are of interest in this study, a slightly different approach was also employed. Each pair of inbreeding levels were analyzed separately by 2-way ANOVA (same model as above) to test for treatment by inbreeding level interaction; 6 ANOVAS were carried out among pairs (0–I, 0–II, 0–III, I–II, I–III, II–III). The presence of interaction with only 2 inbreeding levels in the model indicates that those particular levels differ in their plasticity. Other approaches which partition the interaction sum of squares derived from the full model provide tests only for comparison of an individual genotype's interaction variance with the average response of all genotypes (Plaisted and Peterson 1959; Shukla 1972; Kang and Miller 1984) and are not used here.

Interaction due to differences in plastic response can arise for two reasons: differences in the direction or pattern of response to the treatments or differences in the amount of responsiveness to treatments (Allard and Bradshaw 1964; Schlichting and Levin 1984). If the paired ANOVA indicated the presence of interaction the data were further analyzed to determine whether differences in amounts or patterns of plasticity (or both) were the cause of inbreeding level by treatment interaction. All characters which had a probability for the interaction term of less than 0.15 were analyzed in the following manner. The relative amount of plastic response of the character for the two inbreeding levels was measured by the Coefficient of Variation of treatment means (CV = 100 × standard deviation of treatment means/grand mean of treatments). The CV is a quantitative measure of the relative responsiveness of the character to the 6 treatments. Differences in the pattern of plastic response between inbreeding levels were investigated through the use of the nonparametric Spearman rank correlation (Hollander and Wolfe 1973) between the 6 treatment means for the two inbreeding levels. If the patterns of response are identical Spearman's rho will equal 1.00. If inbreeding changes the direction of response to treatments, correlations between levels will be low or even negative. The CVs and Spearman correlations were computed using the Least Squares means derived from the overall two-way ANOVA (SAS 1982).

Some examples will illustrate the use of the CVs and Spearman correlation to investigate the causes of interaction between inbreeding level and treatments. The character days to flowering shows a significant inbreeding level by treatment interaction term ($P = 0.046$) for inbreeding levels 0 and I. The treatment means are 38, 29, 25, 32, 29, 19 for inbred level 0 and 27, 29, 28, 31, 35, 14 for inbred level I. The amount of plastic response for these two levels is similar (CV = 22 for level 0 and CV = 26 for level I) but the Spearman correlation of rho = 0.32 indicates different patterns of plastic response by the two inbreeding levels. The interaction is significant for the character capsule production for inbred levels 0 and II (treatment means: level 0 = 67, 30, 32, 94, 44, 37; level II = 41, 34, 17, 42, 38, 33). In this case the Spearman correlation is rho = 0.83 suggesting that the patterns of plastic response are similar, but the CVs are quite distinct, 50 for inbred level 0 and 27 for level II.

Results

Treatment responses

Ten of the 12 characters showed highly significant responses to the treatments (Table 1) suggesting that there is a substantial degree of phenotypic plasticity in Dwarf Crimson Beauty just as there is in wild *Phlox drummondii* (Schlichting and Levin 1984). The ranges of character mean values for treatments within each inbreeding level are given in Table 2.

The Early Leaf Removal treatment was generally indistinguishable from the Control treatment for most characters. These two treatments produced the largest plants, with the Control plants producing slightly fewer

Table 1. Probability values for treatment, inbreeding level and their interaction from the results of a two-way analysis of variance for 6 treatments and 4 inbreeding levels in *Phlox drummondii* cultivar 'Dwarf Crimson Beauty'. The full model analyzed was Character = Treatment Inbreeding level Inbreeding level × Treatment Family Block. Probabilities were derived from Type III sums of squares (SAS 1982)

Character	ANOVA effect		
	Treatment	Inbreeding level	Inbreeding level × treatment
Shoot weight	0.0001	0.0009	0.25
Root weight	0.0001	0.002	0.64
Root/shoot	0.0001	0.34	0.16
Plant height	0.0001	0.023	0.0005
Total branches	0.0001	0.004	0.49
Basal/total br.	0.22	0.011	0.73
Internode length	0.0001	0.07	0.27
Total flowers	0.0001	0.0001	0.40
Flowers/gm	0.0001	0.0009	0.28
% selfing	0.73	0.002	0.87
Capsules	0.0001	0.0001	0.19
Days to flower	0.0001	0.18	0.52

flowers and capsules. Control plants also flowered later. Late Leaf Removal had a deleterious effect on plants compared to the Early Leaf Removal with reductions in biomass, flower and capsule production and an increase in the number of days to first flowering. Plants in the Low Water treatment were much shorter and weighed less but had an increased root/shoot ratio compared to those in the Control treatment. The Low Water plants flowered earlier but produced fewer total flowers and capsules.

Plants in the Low Nutrient and Small Pots treatments were stressed the most, with nearly identical average heights and shoot weights. Small Pot plants had somewhat higher root/shoot ratios than the Control (0.18 vs 0.16), and had much higher flowers/gm despite producing very few total flowers and capsules. Branch production and internode length were reduced for Small Pot plants relative to the Control, and flowering commenced an average of 19 days earlier. Low Nutrient plants had the lowest flower and capsule productions among the treatments, and had greatly increased root/shoot ratios (0.31 vs 0.16 for Control).

Table 2. Character means and ranges of mean treatment responses for each inbreeding level in 'Dwarf Crimson Beauty'

Character		Inbreeding level			
		Outcrossed	Inbred I	Inbred II	Inbred III
Shoot weight	mean	2.87	2.75	2.59	2.36
	range	1.50–4.25*	1.38–3.49*	1.24–3.83*	1.23–3.43*
Root weight	mean	0.51	0.49	0.46	0.43
	range	0.30–0.69*	0.27–0.61*	0.32–0.68*	0.25–0.56*
Root/shoot ratio	mean	0.19	0.18	0.19	0.20
	range	0.17–0.32*	0.17–0.30*	0.18–0.35*	0.17–0.37*
Plant height	mean	35.7	34.5	33.4	32.7
	range	32.2–46.6*	26.8–45.8*	27.6–38.8*	27.0–40.1
Total branches	mean	4.5	4.4	4.3	3.9
	range	3.1–5.3*	3.2–5.5	3.0–4.8*	2.6–4.1*
Basal/total branches	mean	0.64	0.70	0.55	0.80
	range	0.34–0.66	0.48–0.72	0.28–0.61*	0.50–1.00
Internode length	mean	1.9	1.9	2.3	2.1
	range	2.3–3.0	1.9–3.0	2.2–3.9	2.2–3.8*
Total flowers	mean	136	125	111	100
	range	89–190*	85–148*	68–141*	62–131*
Flowers/gm	mean	46.4	44.9	42.1	41.0
	range	30.2–57.4*	32.0–55.5*	30.5–59.0*	28.5–56.1*
% selfing	mean	35	35	29	25
	range	29–49	31–38	29–35	25–30
Capsules	mean	51	45	34	26
	range	34–98*	31–55	22–44	18–41*
Days to flower	mean	29	27	31	31
	range	22–42*	17–42*	21–42*	21–44*

* Indicates significant treatment responses within inbreeding level

Total branch production was greatly reduced from the Control (3.1 vs 4.7 branches) and internode distances were increased. Low Nutrient plants flowered a week earlier than Controls.

Inbreeding depression

Inbreeding depression was manifested as reductions in both plant vigor and fecundity (Table 2). Both shoot and root weight and flower production declined with each generation of inbreeding, and capsule production was affected even more with a nearly 50% reduction from the outcrossed plants to the three generation inbred plants (Table 2). Other characters declining significantly with inbreeding include plant height, total branches and flowers/gm. Root/shoot ratios, days to flowering and internode length were not affected by inbreeding.

Treatment × inbreeding interaction

Only one character, plant height, had a significant treatment × inbreeding level interaction in the full model 2-way ANOVA (Table 1). Results of the paired inbreeding level 2-way ANOVAs revealed 5 interactions $P < 0.05$ and an additional 6 comparisons with $P < 0.15$ (Table 3). These 11 characters were investigated for the cause of the interaction using the coefficient of variation (CV) measuring amounts of plastic response, and the Spearman correlation measuring similarity of patterns of plastic response. In 8 of 11 cases the correlations between the treatment responses of the pair of inbreeding levels was > 0.65 indicating general similarity of patterns of plastic response. In 4 of these 8 cases the interaction is clearly due to the differences in the amounts of plastic response to the treatments (CVs, Table 3). For example, in the comparison of capsule production between inbreeding levels 0 and II, the correlation of patterns of response is 0.83 indicating very similar responses to the treatments, but the CVs measuring the amount of plastic response are quite distinct (inbred 0, CV = 50; inbred II, CV = 27). In other instances the interaction may be due to differences in both patterns and amounts of response (e.g. basal/total inbred levels I and II; plant height I and II). Other cases indicate differences in pattern of plastic response as the source of the interaction (e.g. both days to flower and total branches for inbred levels 0 and I). For 0–III shoot, neither patterns or amounts of plasticity seem to account for the level of interaction.

There is no evidence for systematic changes in amounts of phenotypic plasticity due to inbreeding or outcrossing. There is no indication of strong differences in plasticity from the interaction terms with only 5 of the 72 interaction terms significant at $P < 0.05$. Also, those 11 characters for which treatment × inbreeding level interaction was indicated, show no tendency for amounts of plasticity to be greater for more inbred material; CVs decreased with inbreeding 5 times and increased 6 times.

Discussion

Despite a progressive decline in vigor and fecundity with inbreeding, neither amounts or patterns of phenotypic plasticity changed substantially in 'Dwarf Crim-

Table 3. Analysis of cases of interaction observed in the two-way ANOVAs performed for pairs of inbreeding levels. Coefficients of Variation (CV) measure the amount of plastic response to treatments; correlations measure the similarity of patterns of plastic response of the pair of inbreeding levels across treatments

Inbreeding		Character	P[a]	CV level A	CV level B	Spearman correlation
Level A	Level B					
O	I	Capsules	0.11	50	23	0.66
O	I	Total branches	0.13	16	18	0.31
O	I	Days to flower	0.046	22	26	0.32
O	II	Root/shoot	0.15	32	38	0.65
O	II	Capsules	0.05	50	27	0.83
O	III	Shoot weight	0.09	44	40	0.94
O	III	Capsules	0.02	50	35	0.77
I	II	Plant height	0.028	20	14	0.71
I	II	Basal/total	0.13	14	22	−0.31
I	III	Root/shoot	0.04	28	40	0.77
I	III	Flowers/gm	0.10	19	24	0.81

[a] P is the probability associated with the inbreeding level × treatment interaction term from the paired two-way ANOVA

son Beauty'. The results indicate that inbreeding does not increase the amount of phenotypic plasticity and are in accord with the results from studies on *Allium* (Dowker and Fennell 1981) and *Gossypium* (Quisenberry and Kohel 1971). In contrast, results from *Lycopersicon* (Lewis 1954) and *Arabidopsis* (Pederson 1968) have shown a positive relationship between degree of inbreeding and plasticity. A number of other studies (e.g. Griffing and Langridge 1963; Sentz et al. 1954; Williams 1960) have documented the plasticity of hybrids between inbred lines in response to varied environments, but they do not specifically address the issue of the effect of inbreeding per se.

No previous studies have examined the effect of inbreeding on the direction of response of characters to environments, although it would be quite important if inbreeding were to systematically disrupt a genotype's typical responses. Our results indicated that there were only a few instances where differences in the patterns of plastic response were responsible for significant inbreeding level × treatment interaction.

It should be noted here that the results of the full model two-way ANOVA showed significant interaction for only the character plant height. In contrast the paired two-way ANOVAs indicated significant pairwise inbreeding level by treatment interactions for an additional 3 characters: root/shoot ratio, capsule production (twice) and days to flower. There was a significant interaction in only 1 of the 6 pairwise ANOVAs for plant height. These results underscore the differences between the interpretation of the interaction term when there are just two inbreeding levels or greater than two inbreeding levels. The practice of comparing the responses of individual genotypes to the average response of all genotypes is quite common and useful in a variety of circumstances (Finlay and Wilkinson 1963; Eberhart and Russell 1966; Plaisted and Peterson 1959; Shukla 1972; Tai 1979; Kang and Miller 1984). However, if the question of interest involves the comparison of one genotype to another then the method of two-way analysis of variance for individual pairs of genotypes is preferred. Using this method, significant or suggestive interaction terms can be further analyzed to determine whether amounts or patterns of plastic response differ between the genotypes.

Genetically-based differences between wild populations and between cultivated varieties of *P. drummondii* for both the amounts and patterns of plastic response have been documented, and these differences between taxa are unrelated to heterozygosity (Schlichting and Levin, unpublished). Such differences in plasticity also were observed between 3 species of *Phlox* with the least heterozygous species, the facultatively inbreeding *Phlox cuspidata,* no more plastic than the other 2 species (Schlichting and Levin 1984). The available information thus suggests that there is not necessarily a relationship between phenotypic plasticity and inbreeding, and in *Phlox,* even inbreeding serious enough to result in depression of both vigor and yield did not result in changes in phenotypic plasticity.

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References

Allard RW, Bradshaw AD (1964) Implications of genotype-environmental interactions in applied plant breeding. Crop Sci 4:503–508

Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Adv Genet 13:115–155

Dowker BD, Fennell JFM (1981) The relative performance of inbreds and open-pollinated populations of spring-sown onions. J Agric Sci 97:25–30

Eberhart SA, Russell WA (1966) Stability parameters for comparing varieties. Crop Sci 6:36–40

Finlay KW, Wilkinson GN (1963) The analysis of adaptation in a plant-breeding programme. Aust J Agric Res 14: 742–754

Griffing B, Langridge J (1963) Phenotypic stability of growth in the self-fertilized species, *Arabidopsis thaliana.* In: Statistical genetics and plant breeding. NAS-NRC Publ 982:368–394

Hollander M, Wolfe DA (1973) Nonparametric Statistical Methods. Wiley and Sons, New York

Kang MS, Miller JD (1984) Genotype × environment interactions for cane and sugar yield and their implications in sugarcane breeding. Crop Sci 24:435–440

Lerner IM (1954) Genetic homeostasis. Dover Publ Inc, NY

Levin DA (1970) Developmental instability and evolution in peripheral isolates. Am Nat 104:343–353

Levin DA (1975) Gametophytic selection in *Phlox.* In: Mulcahy DL (ed) Gamete competition in plants and animals. North Holland/American Elsevier, Amsterdam NY, pp 207–218

Lewis D (1954) Gene-environment interaction: a relationship between dominance, heterosis, phenotypic stability and variability. Heredity 8:333–356

Pederson DG (1968) Environmental stress, heterozygote advantage and genotype-environment interaction in *Arabidopsis.* Heredity 23:127–138

Plaisted RL, Peterson LC (1959) A technique for evaluating the ability of selections to yield consistently in different locations or seasons. Am Potato J 36:381–385

Quisenberry JE, Kohel RJ (1971) Phenotypic stability of cotton. Crop Sci 11:827–829

Rendel JM (1967) Canalisation and gene control. Academic Press, London

SAS User's Guide: Statistics (1982) SAS Institute, Cary NC
Schlichting CD, Levin DA (1984) Phenotypic plasticity of annual *Phlox:* tests of some hypotheses. Am J Bot 71: 252–260
Sentz JC, Robinson HF, Comstock RE (1954) Relation between heterozygosis and performance in maize. Agron J 46:514–520
Shukla GK (1972) Some statistical aspects of partitioning genotype-environmental components of variability. Heredity 29:237–25
Soule ME (1982) Allomeric variation. 1. The theory and some consequences. Am Nat 120:751–764
Tai GCC (1979) Analysis of genotype-environment interactions of potato yield. Crop Sci 19:434–438
Williams W (1960) Relative variability of inbred lines and F1 hybrids in *Lycopersicum esculentum.* Genetics 45: 1457–1465