
Experimental Evidence for Beneficial Fitness Effects of Gene Flow in Recently Isolated Populations

DARA NEWMAN AND DAVID A. TALLMON*

Division of Biological Sciences, University of Montana, Missoula, MT 59812, U.S.A.

Abstract: *A rich theory has been developed to explain the evolution of populations at equilibrium conditions of gene flow, inbreeding, and selection. There are, however, few empirical examples of the effects of gene flow into recently isolated, small populations under nonequilibrium conditions, such as are expected following population fragmentation. We studied the effects of inbreeding and gene flow in small, experimental populations of the mustard *Brassica campestris* (rapa). Replicate populations of five individuals randomly mated in a growth room received treatments of 0, 1, or 2.5 migrants each generation. Plants from the sixth experimental generation were planted in an outdoor common garden to evaluate the effects of the treatments on fitness and the distribution of phenotypic variation. Regression of six fitness components on inbreeding coefficients indicated a negative effect of inbreeding on fitness for five of these components. The 0-migrant treatment had significantly lower fitness than the migrant treatments for four of six fitness components, but fitness did not differ between the 1-migrant and 2.5-migrant treatments. Phenotypic divergence among populations decreased with an increased number of migrants. These data provide empirical evidence of the beneficial fitness effects of a small number of migrants for recently fragmented populations.*

Evidencia Experimental de los Efectos Benéficos del Flujo de Genes en la Adaptabilidad de Poblaciones Recientemente Aisladas

Resumen: *Una teoría enriquecedora ha sido desarrollada para explicar la evolución de las poblaciones en condiciones de equilibrio de flujo de genes, endogamia y selección. Hay, sin embargo, pocos ejemplos empíricos de los efectos del flujo de genes entre poblaciones recientemente aisladas, pequeñas y bajo condiciones de desequilibrio, tales como las observadas después de una fragmentación poblacional. Estudiamos los efectos de la endogamia y el flujo de genes en poblaciones pequeñas experimentales de la mostaza *Brassica campestris* (rapa). Poblaciones replicadas de cinco individuos cruzadas aleatoriamente en un cuarto de crecimiento recibieron tratamientos de 0, 1 y 2.5 migrantes en cada generación. Las plantas de la sexta generación experimental fueron plantadas en un jardín común externo para evaluar los efectos de los tratamientos en la adaptabilidad y la distribución de la variación fenotípica. La regresión de seis componentes de la adaptabilidad con los coeficientes de endogamia indicó un efecto negativo de la endogamia en la adaptabilidad para cinco de estos componentes. El tratamiento de 0 migrantes tuvo significativamente menos adaptabilidad para cuatro de los seis componentes medidos que los tratamientos con migrantes, pero la adaptabilidad no difirió entre los tratamientos con 1 y 2.5 migrantes. La divergencia fenotípica entre poblaciones disminuyó entre poblaciones con un número creciente de migrantes. Estos datos proveen evidencias empíricas de los efectos benéficos de un pequeño número de migrantes en la adaptabilidad de poblaciones recientemente fragmentadas.*

Introduction

Habitat fragmentation increases the risk of species extinction because it results in loss of habitat and increased

*email datum@selway.umt.edu

Paper submitted February 28, 2000; revised manuscript accepted November 8, 2000.

isolation of existing habitat patches. It has been shown that both demographic and genetic factors can increase extinction risk for species that are fragmented into a number of small populations (Newman & Pilon 1997; Groom 1998; Saccheri et al. 1998). Gene flow can potentially increase the fitness of small, recently fragmented populations, but this idea has received little em-

pirical testing (Ellstrand & Elam 1993; Schemske et al. 1994) and is based largely on single-locus genetic theory, which unrealistically assumes equilibrium conditions. A dearth of experimental tests has led to a lack of understanding of the genetic changes that occur in small populations following fragmentation (i.e., nonequilibrium conditions). We present data showing that a small amount of gene flow among recently inbred, small populations increases the fitness of these populations and that higher rates of gene flow may increase fitness but will also decrease phenotypic divergence among populations.

Gene flow, genetic drift, and selection determine the distribution of genetic variation within and among populations. These forces may often act in opposing directions, and theory predicts various outcomes depending on how they interact (Allendorf 1983). Small populations are particularly vulnerable to the effects of genetic drift, inbreeding, and decreased genetic variation. Inbreeding is often accompanied by inbreeding depression—a reduction in fitness. Numerous studies report a reduction in survival and fecundity due to inbreeding (Charlesworth & Charlesworth 1987; Kalisz 1989; Barrett & Kohn 1991; Johnston 1992; Dole & Ritland 1993; Molina-Freaner & Jain 1993; Willis 1993; Nason & Ellstrand 1995; Koelewijn 1998). Ultimately, inbreeding depression has increased the probability of population extinction in simulated (Mills & Smouse 1994), experimental (Newman & Pilson 1997), and natural (Saccheri et al. 1998) populations.

Theory predicts that gene flow can potentially eliminate the fitness reduction associated with drift and inbreeding in small populations by introducing genetic variation. Wright (1931) analyzed inbreeding coefficients in a large number of finite populations and suggested that a small number of migrants among populations each generation is sufficient to avoid inbreeding depression. Specifically, he showed that the exchange of one migrant per generation among populations maintains the same alleles in all populations at equilibrium (Kimura & Ohta 1971). Fixation of alleles within populations is prevented, but allele frequencies may still diverge among populations (Wright 1951; Kimura & Maruyama 1971; Kimura & Ohta 1971; Mills & Allendorf 1996), allowing local adaptation to occur while inbreeding depression is avoided. This theory, however, is based entirely on consideration of a single locus at drift-migration equilibrium. Most fitness traits are influenced by many loci with complex interactions among them and strong genotype-by-environment interactions (e.g., Shook & Johnson 1999). Therefore, although allele frequencies in small populations sharing migrants may closely follow predictions of single-locus theory, the fitness effects of gene flow are much more difficult to predict and have not been addressed for small populations of plants.

Lande (1992) extended Wright's findings to an explicit consideration of the effects of migration and muta-

tion on additive genetic variance in subdivided populations. He found that the equilibrium level of total additive genetic variance in subdivided populations is equal to that in a panmictic population of the same total effective size, regardless of the migration rate. These results apply, however, to equilibrium conditions in a large number of small populations. As a result of human-caused habitat fragmentation, nonequilibrium conditions will prevail, and usually there will be only a small number of populations. For a small number of populations that have been isolated recently, inbreeding depression over the short term and the accumulation of deleterious mutations over many generations are likely to decrease fitness and increase extinction risk (Lynch et al. 1994). Consequently, some level of migration may be necessary to maintain adaptive variation in small, recently isolated populations.

How many migrants are needed to eliminate inbreeding effects? Current models are inadequate to make predictions about the fitness effects of migration because natural populations of conservation concern will not fit assumptions of the aforementioned theories. Often, there will be only a few populations and they will usually have small effective sizes. Clearly, empirical data are needed to determine the effectiveness of different levels of migration in maintaining the fitness of small, recently fragmented populations threatened with immediate extinction.

Unfortunately, empirical evidence for the role of migrants in preventing inbreeding depression in small populations is scarce and sometimes conflicting (Ellstrand & Elam 1993; Schemske et al. 1994), and experiments have been limited to laboratory populations of insects. For example, fitness increased in low-fitness populations of *Tribolium* following the introduction of migrants from high-fitness populations (Wade & Goodnight 1991) but not following the introduction of migrants from randomly selected populations. Spielman and Frankham (1992) found that fitness increased in highly inbred laboratory lines ($F = 0.50$) of *Drosophila* following the treatment of one migrant per generation. In two studies by the same research group, one migrant per generation among inbred laboratory populations of *Musca* failed to increase fitness over a few generations (Backus et al. 1995) but did increase fitness in a longer experiment (Bryant et al. 1999).

In a study of natural populations, Storfer and Sih (1998) found a negative correlation between numbers of migrants estimated from allozyme data and fitness components in streamside salamander (*Ambystoma barbouri*) populations with opposing selection pressures. Nevertheless, it is unclear in this study, as in most studies that infer gene flow from the distribution of molecular variation, whether estimated levels of gene flow reflect ongoing or historical events (Whitlock & McCauley 1999). Consequently, the amount of migration necessary to avoid both inbreeding and outbreeding depression in small populations remains an important unanswered question.

Plant studies showing the positive effects of crossing inbred individuals to unrelated individuals are not uncommon, but none have experimentally manipulated migration levels among populations to see whether mean population fitness can be increased. For example, Heschel and Paige (1995) showed that crosses of 10 individuals from each of two small populations with individuals from distant populations increased fitness, but they did not examine the effects of different levels of gene flow into their small populations. Our study is the first we know of to experimentally investigate the fitness and phenotypic effects of specific levels of migration among small plant populations.

Our experimental approach allowed us to control migration rates and avoid the problems associated with migration-rate estimates derived from molecular-marker data. We investigated the role of varying numbers of migrants in maintaining population fitness and its effects on the distribution of phenotypic variation within and among populations. We addressed three specific questions important to the evolution and conservation of small populations: (1) Do migrants decrease the detrimental effects of inbreeding? (2) If so, are more migrants better than fewer? (3) How do migrants affect the distribution of phenotypic variation between and within populations?

Methods

Experimental Organism and Site

Brassica campestris (*rapa*) is a self-incompatible annual species of the mustard (Brassicaceae) family introduced to, and common throughout, North America (Lackschewitz 1991). Populations range in size from a few to thousands of individuals. Numerous yellow flowers are borne on raceme inflorescences producing approximately 15 seeds per capsule. The seeds used to begin this experiment were collected from an established population of several thousand individuals growing along an irrigation ditch in the Bitterroot Valley south of Missoula, Montana.

Experimental Design

To test the effectiveness of different numbers of migrants in reducing the detrimental effects of inbreeding, we performed a two-way factorial experiment with three treatments and 10 blocks (Fig. 1). Thirty populations were founded with five seeds, one taken from each of five unrelated maternal sibship families and raised in a growth room at the University of Montana with constant light. For five generations, three levels of gene flow from a migrant pool—0, 1, or 2.5 migrants each generation—served as treatments. Seeds produced by fifth-generation plants were planted at the University of Montana garden

Experimental Design

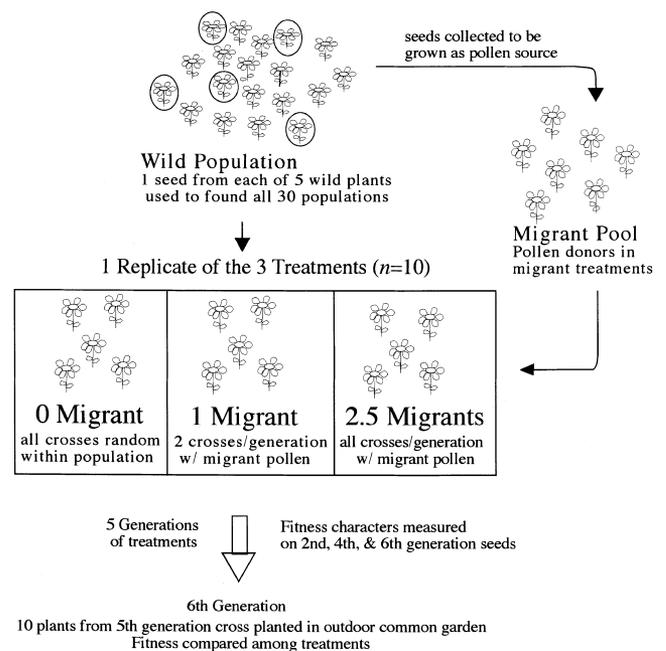


Figure 1. Experimental design used to assess the effects of 0, 1, and 2.5 migrants per generation on the fitness of *Brassica campestris* populations. Each population was founded with one seed from each of five wild individuals. Populations of five individuals received migration treatments for five generations in a growth room. Fitness was assessed for sixth-generation plants grown in an outdoor common garden.

plots, and fitness data were collected from these individuals after they matured.

Five pairs of plants within each population were randomly chosen each generation to produce the five seeds for the subsequent generation. No self-pollination was permitted. Artificial pollination was performed with the floral buds to ensure that no previous pollination had occurred. Four to seven fully developed buds on the selected dam were opened and emasculated. The stigma was dusted with a thick layer of pollen from a randomly selected sire. Several buds were pollinated to ensure adequate seed production.

In the 1-migrant treatment, two of the five sires were chosen randomly from the migrant pool. Migrant number is the number of individuals reproducing in a population that come from another population (Wright 1951). Therefore, two pollen donors constitute one individual migrant because they each contribute half the genome of their offspring. Accordingly, all five crosses were performed with migrant pollen for the 2.5-migrant treatment. The seeds for the migrant pool were selected from the same natural population as the five founder families

and grown to produce a source of pollen for the migrant pool. The maternal plants for the migrants were placed a minimum of 1 m apart and a minimum of 5 m from the founder families. We assumed that the migrants were unrelated to both the original families and to each other ($F = 0$) but were genetically similar enough to avoid outbreeding depression.

The initial generation was planted in August 1992. One seed from each of the five crosses per population for each of the 30 populations was planted to initiate each subsequent generation. Approximately two generations per year were completed. The fifth generation was planted in December 1994, and the resulting seeds were harvested in March 1995. The seeds from this generation were planted in the outdoor common garden. Three of the 30 populations had an inadequate number of seeds by the sixth generation, so seeds from the fifth generation of these three populations were planted in the field. This was not a problem because we kept a pedigree that allowed us to calculate the inbreeding coefficients of all individuals.

Ten seeds from each of the five crosses per population, instead of a single seed as was used in all previous generations, were planted in the field to produce the sixth generation. In each block, 150 seeds (10 seeds per cross \times 5 crosses per population \times 3 treatments per block) were planted in a randomized design. On 22 April 1995, 1500 seeds (150 seeds per block \times 10 blocks) were germinated in 96-cell flats in the growth room. One week later, plants were transferred to field plots free of vegetation. Two blocks were combined into an 80 \times 60 cm plot, and each plant was assigned to a unique 4-cm² cell. Plants that died from transplant shock were replaced, when replacements were available, for 2 weeks following the initial planting. Plants were watered weekly or bimonthly, depending on weather conditions, over the course of the summer. Plants began to senesce at the end of July and were harvested on 11 August 1995.

Inbreeding Coefficients

We used a pedigree to calculate inbreeding coefficients (F) of all individuals so that we could examine the relationship between fitness, inbreeding, and migration levels. We calculated the expected F for each treatment each generation with equations derived by Wright (1969, 1977) and adjusted for the case where selfing is precluded (Crow & Denniston 1988). The expected F each generation was calculated for the 0-migrant treatment with the equation

$$F_t = 1 - (1 - 1/(2N + 1))^t,$$

where t is the generation and N is the genetically effective population size of 5 (Wright 1977). The expected F each generation for the 1-migrant treatment was calcu-

lated with Wright's (Wright 1969) island model equation

$$F_t = (1 - m)^2((1/2N) + (2N/2N + 1)F_{t-1}),$$

where m is the proportion of exchange between populations. The equilibrium F for the 2.5-migrant treatment is zero because all plants were pollinated by unrelated sires from the migrant pool.

Fitness

We tested the effects of migrants on fitness in the lab by measuring several fitness characters on seeds that developed into the second-, fourth-, and sixth-generation plants. Germination rate was determined by the number of seeds out of 10 planted that germinated within 1 week. The number of seeds per capsule and weight per 10 seeds were determined for the second- and fourth-generation seeds only. Fixed effects two-way analysis of variance (ANOVA) was used, with block effect and treatment effect as the two factors, to test for differences among seeds from the three treatments in germination rate, seed weight, and number of seeds per capsule.

A more rigorous test of the effects of migrants on fitness examines six plant fitness traits in an outdoor environment. Cotyledon width, initial flowering date, stem diameter (at base), aboveground dry weight, seed number, and seed weight were measured on sixth-generation individuals transplanted outdoors. These traits have been used in previous studies as measures of plant fitness (Harper 1977; Grime 1979). In addition to these fitness components, we measured a characteristic unlikely to be a component of fitness, leaf shape. Leaf length and width were measured on the third leaf from the ground, and leaf shape was estimated by dividing the former by the latter. The mean value for each character was compared among treatments with the same ANOVA used in the laboratory tests. Each family value was a mean of the 10 full-siblings in that plot, and the mean value for each plot was a mean of the five families in that plot. Each treatment was represented only one time per block ($n = 10$ blocks), which prevents the analysis of a treatment-by-block interaction. Arcsine square-root transformation was required to meet the assumption of normality for germination rate. Bonferroni corrections were performed to prevent inflated Type I errors due to multiple comparisons. Linear regressions were used to test for a significant relationship between each of the characters measured and the inbreeding coefficients.

Phenotypic Variation

We examined the effects of migrant treatments on among-population phenotypic divergence in morphology as an

index of the extent to which gene flow facilitates or prevents divergence among populations (i.e., local adaptations). Specifically, a random-effects ANOVA was performed on each treatment separately for each character to test for significant variation due to differences between groups (i.e., variation among the 10 populations in each treatment). The mean square error represents the mean within-population variation (V_w), and the between-population variation (V_b) is calculated as the mean-square group minus the mean-square error divided by the sample size of 10 (Sokal & Rohlf 1981). Therefore, the proportion of total phenotypic variation (V_T) due to between-population divergence can be calculated as V_b/V_T . The distributions of phenotypic variation in both character and residual values were analyzed. Residual values were determined by grouping all 150 values together within a plot and using block as the single factor. In this way, the environmental effects between blocks were removed. Because treatment-by-block interactions may have been present for some characters measured, but could not be tested with our experimental design, character values are also presented to describe the distribution of phenotypic variation.

Results

Inbreeding Coefficients

The mating design significantly increased levels of inbreeding, closely following expectations. The mean F calculated each generation from the 0-migrant pedigree was similar to that predicted with the equation

$$F_t = 1 - (1 - 1/(2N + 1))^t,$$

and the observed F for the 1-migrant treatment mirrored closely the values predicted with the equation

$$F_t = (1 - m^2)(1/(2N + 1) + 2N/(2N + 1)F_{t-1})$$

(Fig. 2). At the conclusion of the experiment, the observed values of $F = 0.33$, $F = 0.08$, and $F = 0.00$ for the 0-, 1-, and 2.5-migrant treatments, respectively, were similar to their respective predicted values of $F = 0.32$, $F = 0.12$, and $F = 0.00$. The mean F of the 0- and 1-migrant treatments would have continued to increase up to equilibrium values of 1.00 and 0.15, respectively, had the experiment been continued for more generations.

Fitness

The seed characters measured in the lab—germination rate, seed size, and seed number per capsule—did not show consistent differences among treatments across generations (Table 1). The only statistically significant difference detected was fewer seeds per capsule in the 0-migrant treatment in the fourth generation, although

germination rate was lowest in the 0-migrant treatment in both the fourth and sixth generations. In the sixth generation, only germination rate was measured.

The effectiveness of migration in reducing inbreeding depression was much more pronounced in the field. Regressions of fitness-character values on F values calculated from experimental pedigrees indicated a significant relationship between F and all fitness components except seed weight per 10 seeds and no significant relationship with the nonfitness component of leaf shape (Fig. 3). Cotyledon width, stem diameter, aboveground dry weight, and seed number per plant were negatively related to F , whereas flowering date increased as F increased.

The field comparison of fitness levels among treatments also provides a clear picture of the importance of gene flow to recently inbred, small populations. The absence of gene flow greatly lowered fitness in the 0-migrant treatment by the sixth generation. In general, the 0-migrant treatment had significantly lower values for the measured characters than did the 1- and 2.5-migrant treatments. In contrast, the 1- and 2.5-migrant treatments did not differ from each other in any fitness characters (Table 2). The 1- and 2.5-migrant treatments showed greater fitness values than the 0-migrant treatment for cotyledon width, seed diameter, dry weight, and seed number. The number of migrants did not significantly affect the nonfitness character of leaf shape, seed weight, or flowering date, although the 1- and 2.5-migrant treatments did flower slightly earlier. Significant block effects were found for many characters measured (Table 2), which suggests the existence of small-scale variation within the physical environment of the outdoor common garden and genotypic differences among blocks. But the data do indicate clear beneficial effects

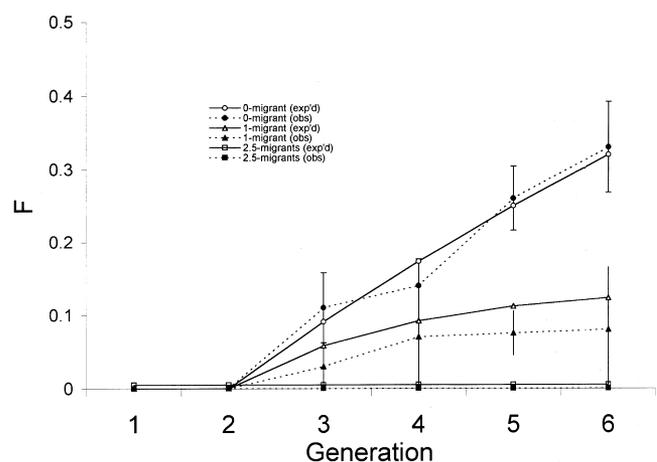


Figure 2. Mean (SD) inbreeding coefficients for the 0-, 1-, and 2.5-migrant treatments calculated from known pedigrees for all *Brassica campestris* plants (obs) compared with expected values (exp'd).

Table 1. Mean values (SE) for seed characters measured in *Brassica campestris* populations receiving 0, 1, and 2.5 migrants each generation.*

Generation	Character		
	germination rate	weight (g)/ 10 seeds	no. of seeds/ capsule
2			
0 migrants	0.95 (2.0)	0.017 (0.0008)	21.1 (1.0)
1 migrant	0.91 (2.7)	0.017 (0.0008)	20.9 (1.0)
2.5 migrants	0.91 (2.7)	0.017 (0.0008)	20.0 (1.5)
4			
0 migrants	0.89 (0.05)	0.019 (0.0009)	17.6 (1.7)a
1 migrant	0.95 (0.02)	0.017 (0.0009)	21.2 (1.2)b
2.5 migrants	0.93 (0.02)	0.019 (0.0009)	24.1 (1.3)b
6			
0 migrants	0.79 (0.07)		
1 migrant	0.94 (0.03)		
2.5 migrants	0.89 (0.04)		

*Significant differences between treatments are represented by different letters (a,b) and are based on least-squares differences tests ($n = 10$; Bonferroni corrected $p < 0.05$). Only germination rate was measured in generation 6.

for the fitness of 1 and 2.5 migrants per generation despite this variation, especially when these effects are considered across all fitness characters measured.

Effects on Phenotypic Variation

The migrant treatments also affected the capacity for populations to diverge morphologically in the field. In general, a greater proportion of total phenotypic variation was due to population divergence in the 0-migrant treatment than in the migrant treatments, and V_b/V_t decreased with increased numbers of migrants (Table 3). For example, 24.4% of the total phenotypic variation in cotyledon width in the total 0-migrant populations (of 50 plants) was due to V_b , whereas V_b did not contribute significantly to V_t in the 2.5-migrant treatment. In cases where character values showed significant between-population divergence across all treatments, the trend toward greater phenotypic divergence at lower migration levels was consistent. For example, 59%, 33%, and 15% of the flowering-date V_t was due to V_b in the 0-, 1-, and 2.5-migrant treatments, respectively, even though all were statistically significant. In general, the coefficients of variation were similar between the character values for the different traits. The coefficients of variation for the residuals were more variable, both between traits and within traits between treatments, than they were for the character values.

Discussion

The 0-migrant treatment had significantly lower mean fitness values than the migrant treatments. No fitness dif-

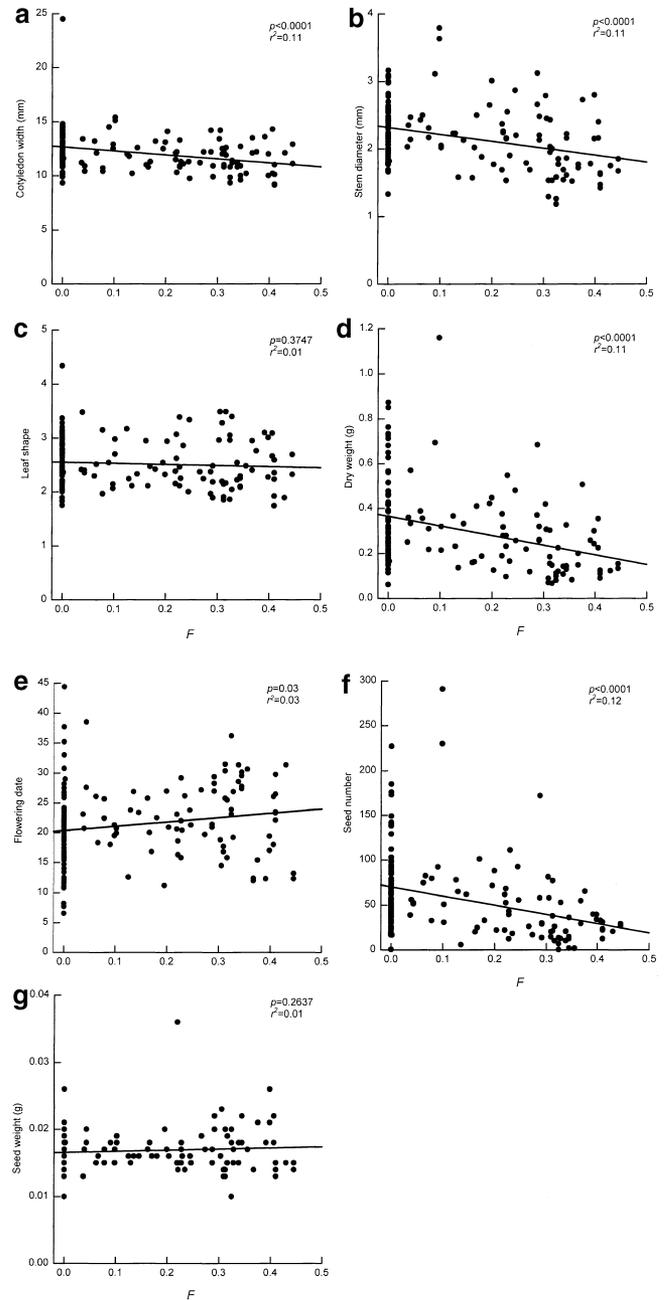


Figure 3. Linear regression of (a-g) fitness characters on inbreeding coefficient, F , for all sixth-generation *Brassica campestris* plants.

ferences were detected between the 1- and 2.5-migrant treatments. These results support the idea that a single migrant per generation can play an important role in preventing the detrimental effects of inbreeding due to small population size and is sufficient to maintain genetic variation and fitness in small populations over the short term. In addition, we demonstrated that a decrease in the proportion of phenotypic variation due to population divergence accompanies an increase in the number of migrants.

Table 2. Mean values (SE) for six fitness characters and the nonfitness component of leaf shape measured for sixth-generation *Brassica campestris* plants from populations receiving 0, 1, or 2.5 migrants per generation.*

Character	Migration level per generation			Effects (p)	
	0	1	2.5	treatment	block
Cotyledon width (mm)	11.4 (0.32)	12.3 (0.30)	12.8 (0.30)	<0.0005	<0.0005
Stem diameter (mm)	1.96 (0.12)	2.29 (0.12)	2.29 (0.10)	<0.005	<0.0005
Leaf shape	2.52 (0.11)	2.50 (0.10)	2.54 (0.11)		
Dry weight (g)	0.21 (0.03)	0.36 (0.06)	0.35 (0.04)	<0.005	<0.0005
Flowering date (days)	23.1 (1.8)	21.0 (1.3)	19.9 (1.5)		
Seed number	34.4 (7.0)	65.5 (12.9)	68.8 (10.2)	<0.005	<0.005
Seed weight (g)	0.017 (0.001)	0.017 (0.0003)	0.016 (0.0003)		<0.05

*For all characters with significant treatment effects, the 1- and 2.5-migrant treatments are significantly different from the 0-migrant treatment but not from each other, as determined by least-squares differences tests. Bonferroni-corrected levels of significance are shown.

Although the natural world is much more complicated than the experimental design we used, our results provide insight into theoretical predictions of the interactions among the evolutionary forces that determine the fate of small populations. Our results support predictions derived largely from relatively simple models of single-locus population genetics and inbreeding coefficients developed by Wright (1932), despite the fact that many of the assumptions of these models were violated. Specifically, the beneficial effects of gene flow were seen in just a few generations following inbreeding, in a small number of populations, and before equilibrium levels of inbreeding were reached.

Our results provide definitive experimental evidence for the beneficial role of low numbers of migrants into inbred populations. The 0-migrant treatment had roughly half as many seeds per plant as the 1- and 2.5-migrant treatments (Table 2). Seed number is an estimate of overall fitness for an annual plant, provided that viability is similar between the treatments (Crawley 1986). Because seed weight did not differ among treatments, and because viability is correlated with seed weight in this species (D. Pilson, personal communication), the 0-migrant

treatment had lower overall fitness. Several additional fitness components, including cotyledon width, seed diameter, and dry weight, also indicated significantly lower fitness in the 0-migrant treatment.

The 1- and 2.5-migrant treatments showed similar fitness levels despite having different mean inbreeding coefficients of 0.08 and 0, respectively. The 8% higher F of the 1-migrant treatment is close to the 10% value Frankel and Soulé (1981) found associated with a 5–10% fitness reduction in many taxa. It is possible that the two treatments would have shown fitness differences over more generations as their mean inbreeding coefficients continued to diverge. The significant linear relationships between five fitness components, including seed number, and F , support this possibility.

The proportion of total genetic variation due to genetic divergence among populations is expected to increase as migration decreases (Wright 1951; Lande 1992). Genetic drift has a greater effect on small populations, which results in a greater variance in allele frequencies and genetic divergence than in large populations (Crow & Kimura 1970; Lacy 1987). Empirical studies of rare versus common plants and large versus small plant popula-

Table 3. The distribution of phenotypic variance in *Brassica campestris* fitness characters measured from sixth-generation plants in 0-, 1-, and 2.5-migrant treatments.^a

Character	0 migrant				1 migrant				2.5 migrants			
	residuals		character		residuals		character		residuals		character	
	$V_b/V_t\%$	CV										
Cotyledon width	—	1.42	24.4 ^b	0.12	—	7.10	25.3 ^b	0.11	—	3.13	0.2	0.16
Stem diameter	6.0	1.35	45.8 ^b	0.23	—	3.34	30.0 ^b	0.22	0.8	2.78	30.0 ^b	0.18
Leaf shape	2.4	20.2	23.5 ^b	0.20	4.2	21.5	20.0 ^b	0.18	14.4 ^b	20.2	12.0 ^b	0.16
Dry weight	—	1.03	36.2 ^b	0.61	—	3.44	31.7 ^b	0.67	—	3.67	20.7 ^b	0.53
Flower date	32.2 ^b	2.6	59.2 ^b	0.27	24.1 ^b	15.9	32.6 ^b	0.26	0.5	4.4	14.9 ^b	0.37
Seed number	6.5	1.16	25.1 ^b	0.87	—	4.12	31.4 ^b	0.80	0.9	3.15	19.0 ^b	0.68
Seed weight	12.2 ^b	—	45.2 ^b	0.18	—	—	—	0.18	—	—	14.9 ^b	0.13

^aPercent total phenotypic variance (V_t) due to between-population variance (V_b) is based on residual and character values. Coefficient of variation (CV) allows for comparisons between characters with different means. The V_b/V_t could not be determined when mean square error was greater than mean square group (—).

^bA statistically significant contribution ($p < 0.05$) of V_b to V_t after Bonferroni corrections for multiple tests.

tions support this expectation (Schwaegerle & Schaal 1979; Moran & Hopper 1983; Karron 1988; van Treuren et al. 1991). Studies have shown that within-population phenotypic variation is lower in small plant populations (Ouborg et al. 1991) but have not addressed the distribution of phenotypic variation between and within populations.

Our results are consistent with the expectation of a greater proportion of total phenotypic variation due to between-population divergence when drift is the driving force. As migrants bring in outside variation, the amount of total phenotypic variation due to within population variation increases. This trend is consistent among all characters measured and indicates that phenotypic variation, and possibly genetic variation, may be distributed similarly to allozyme variation—as predicted by theory (Lande 1992) and shown empirically in *Daphnia* (Spitze 1993). Presumably some of this phenotypic variation, which is acted upon by natural selection, has a genetic basis and provides fodder for local adaptation.

A potential problem of a large number of migrants is the loss of local adaptation. Therefore, a balance between population divergence, which allows for local adaptation, and the maintenance of within-population variation, which provides material for future evolution, is desired. Although all treatments showed significant among-population divergence for some characters, levels of divergence were consistently greatest for the 0-migrant treatment, intermediate for the 1-migrant treatment, and lowest for the 2.5-migrant treatment. Because one migrant per generation appeared adequate to reduce the deleterious fitness effects of inbreeding (Table 2), this low level of migration may optimally balance the conflicting consequences of gene flow by supplying needed genetic variation and reducing inbreeding depression while permitting local adaptation. Studies directed at determining how gene flow affects local adaptation are needed to clarify this issue.

There are demographic and genetic reasons why more than one migrant may be necessary to maintain fitness in natural populations (for a review see Mills & Allendorf 1996). For example, if a population has become highly inbred, then an initial pulse of several migrants may be necessary to alleviate inbreeding depression (Hedrick 1995). Or, if migrants have reduced breeding success relative to residents, then more than one migrant may be needed. If populations are strongly locally adapted, however, then the movement of large numbers of migrants can lead to outbreeding depression and increased risk of local population extinction (e.g., Edmands 1999). Thus, both genetic and ecological information will be invaluable in determining the optimal number of migrants for the fitness of natural populations.

Our results are especially pertinent in the context of in situ conservation of plant populations. Although our experimental plant populations were kept small in order

to conduct the experiment over a short time frame, many natural plant populations that receive conservation attention will also be small and subject to inbreeding depression. For example, Ellstrand and Elam (1993) found that over 50% of 743 sensitive plant taxa in California occurred in populations of fewer than 100 individuals. Because the effective size of natural plant populations, which controls the rate of inbreeding, can be as small as 10% of the population size (Husband & Barrett 1992), sensitive plant populations are at a high risk of inbreeding depression. In addition, because many plant species have self-incompatibility systems that prevent successful fertilization by pollen grains of the same mating type as the recipient plant (de Nettancourt 1977), the introduction of pollen from other populations may be vital to maintaining local population fitness. For example, Reinartz and Les (1994) found reduced seed set in one-third of the last 14 Wisconsin populations of *Aster furcatus*, due to a lack of diversity in mating types. Demauro (1993) found the last Illinois population of the lakeside daisy (*Hymenoxys acaulis* var. *glabra*) to be effectively extinct because all individuals were of the same mating type and had produced no seeds for over 15 years. These examples suggest that interpopulation movement of pollen from a small number of individuals may be an important component of effective strategies to maintain genetic diversity and fitness in natural plant populations. In the case of the lakeside daisy, recovery plans include the use of this management tool (Demauro 1993).

Small populations of endangered or threatened species are vulnerable to extinction from factors such as inbreeding and demographic stochasticity. Human manipulation may be essential, in some cases, to maintain viable populations following habitat fragmentation. The question, then, is how should managers move individuals among populations to maintain short- and long-term fitness? Our results suggest that one migrant each generation will maintain the same level of fitness as that found with 2.5 migrants each generation and will still allow for phenotypic divergence between the populations. The rule of one migrant per generation has previously received little testing, and what testing has been done has led to somewhat conflicting results. Our study provides empirical evidence of the effects of genetic drift and gene flow in the evolution of small populations, and insight into management alternatives for conserving small populations.

Acknowledgments

We thank F. W. Allendorf, M. Lindberg, L. S. Mills, D. Patterson, and S. Samuels for helpful suggestions. F. W. Allendorf, D. Emlen, J. McKay, and L. S. Mills also provided helpful comments on earlier versions of this manuscript. We give special thanks to D. Pilson for her sup-

port and guidance. During preparation of this paper we received financial support from the National Science Foundation in the form of a dissertation improvement grant (DEB 9212583) to D.N. and a graduate research training fellowship (DGE 9553611) to D.A.T.

Literature Cited

- Allendorf, F. W. 1983. Isolation, gene flow, and genetic differentiation among populations. Pages 51–65 in C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas, editors. *Genetics and Conservation*. Benjamin/Cummings, Menlo Park, California.
- Backus, V. L., E. H. Bryant, C. R. Hughes, and L. M. Meffert. 1995. Effect of migration or inbreeding followed by selection on low-founder-number populations: implications for captive breeding programs. *Conservation Biology* 9:1216–1224.
- Barrett, S. C. H., and J. R. Kohn. 1991. The application of minimum viable population theory to plants. Pages 3–31 in D. A. Falk and K. Holsinger, editors. *Genetics and conservation of rare plants*. Oxford University Press, New York.
- Bryant, E. H., V. L. Backus, M. E. Clark, and D. H. Reed. 1999. Experimental tests of captive breeding for endangered species. *Conservation Biology* 13:1487–1496.
- Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18:237–268.
- Crawley, M. 1986. The structure of plant communities. Pages 1–50 in M. Crawley, editor. *Plant ecology*. Blackwell Scientific, Oxford, United Kingdom.
- Crow, J. F., and M. Kimura. 1970. *An introduction to population genetics theory*. Burgess Publishing, Minneapolis, Minnesota.
- Crow, J. F., and C. Denniston. 1988. Inbreeding and variance effective population numbers. *Evolution* 42:482–495.
- Demauro, M. M. 1993. Relationship of breeding system to rarity in the lakeside daisy (*Hymenoxys acaulis* var. *glabra*). *Conservation Biology* 7:542–550.
- de Nettancourt, D. 1977. *Incompatibility in angiosperms*. Springer-Verlag, New York.
- Dole, J., and K. Ritland. 1993. Inbreeding depression in two *Mimulus* taxa measured by multigenerational changes in the inbreeding coefficient. *Evolution* 47:361–373.
- Edmands, S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* 53:1757–1768.
- Ellstrand, N., and D. Elam. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* 24:217–242.
- Frankel, O. H., and M. E. Soulé. 1981. *Conservation and evolution*. Cambridge University Press, Cambridge, United Kingdom.
- Grime, J. P. 1979. *Plant strategies and vegetation processes*. Wiley, New York.
- Groom, M. L. 1998. Allee effects limit population viability of an annual plant. *The American Naturalist* 151:487–496.
- Harper, J. L. 1977. *Population biology of plants*. Academic Press, London.
- Hedrick, P. W. 1995. Gene flow and genetic restoration: the Florida panther as a case study. *Conservation Biology* 9:996–1007.
- Heschel, M. S., and K. N. Paige. 1995. Inbreeding depression, environmental stress, and population size variation in scarlet gilia (*Ipomopsis aggregata*). *Conservation Biology* 9:125–133.
- Husband, B. C., and S. C. H. Barrett. 1992. Effective population size and genetic drift in trisylous *Eichhornia paniculata* (Pontederiaceae). *Evolution* 46:1875–1890.
- Johnston, M. 1992. Effects of cross and self-fertilization on progeny fitness in *Lobelia cardinalis* and *L. siphilitica*. *Evolution* 46:688–702.
- Kalisz, S. 1989. Fitness consequences of mating system, seed weight, emergence date in the winter annual, *Collinsia verna*. *Evolution* 43:1263–1272.
- Karron, J. 1988. Genetic structure of populations of geographically restricted and widespread species of *Astragalus*. *American Journal of Botany* 75:1114–1119.
- Kimura, M., and T. Maruyama. 1971. Pattern of neutral polymorphisms in a geographically structured population. *Genetical Research* 18:125–131.
- Kimura, M., and T. Ohta. 1971. *Theoretical aspects of population genetics*. Princeton University Press, Princeton, New Jersey.
- Koelewijn, H. P. 1998. Effects of different levels of inbreeding on progeny fitness in *Plantago coronopus*. *Evolution* 53:692–702.
- Lackschewitz, K. 1991. *Vascular plants of west-central Montana: identification guidebook*. U.S. Department of Agriculture Intermountain Research Station, Missoula, Montana.
- Lacy, R. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection and population subdivision. *Conservation Biology* 1:143–158.
- Lande, R. 1992. Neutral theory of quantitative genetic variance in an island model with local extinction and colonization. *Evolution* 46:381–389.
- Lynch, M., J. Conery, and R. Burger. 1994. Mutation accumulation and the extinction of small populations. *The American Naturalist* 146:489–517.
- Mills, L. S., and F. W. Allendorf. 1996. The one-migration-per-generation rule in conservation genetics and management. *Conservation Biology* 10:1509–1518.
- Mills, L. S., and P. E. Smouse. 1994. Demographic consequences of inbreeding in remnant populations. *The American Naturalist* 144:412–431.
- Molina-Freaner, F., and S. Jain. 1993. Inbreeding effects in a gynodioecious population of the colonizing species *Trifolium birtum*. *Evolution* 47:1472–1479.
- Moran, G., and S. Hopper. 1983. Genetic diversity and insular population structure of rare granite rock species, *Eucalyptus caesia*. *Australian Journal of Botany* 31:161–172.
- Nason, J., and N. Ellstrand. 1995. Lifetime estimates of biparental inbreeding depression in the self-incompatible annual plant *Raphanus sativus*. *Evolution* 49:307–316.
- Newman, D., and D. Pilson. 1997. Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evolution* 51:354–362.
- Ouborg, N. J., R. van Treuren, and J. M. van Damme. 1991. The significance of genetic erosion in the process of extinction. II. morphological variation and fitness components in populations of varying size of *Salvia pratensis* and *Scabiosa columbaria*. *Oecologia* 86:359–367.
- Reinartz, J. A., and D. H. Les. 1994. Bottleneck-induced dissolution of self-incompatibility and breeding system consequences in *Aster furcatus* (Asteraceae). *American Journal of Botany* 81:446–455.
- Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392:491–494.
- Schemske, D., B. Husband, M. Ruckelshaus, C. Goodwillie, I. Parker, and J. Bishop. 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* 75:586–606.
- Schwaegerle, K., and B. Schaal. 1979. Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea*. *Evolution* 33:1210–1218.
- Shook, D. R., and T. E. Johnson. 1999. Quantitative trait loci affecting survival and fertility-related traits in *Caenorhabditis elegans* show genotype-environment interactions, pleiotropy and epistasis. *Genetics* 153:1233–1243.
- Sokal, R., and J. Rohlf. 1981. *Biometrics*. Freeman, New York.
- Spielman, D., and R. Frankham. 1992. Modeling problems in conservation genetics using captive *Drosophila* populations: improvement of reproductive fitness due to immigration of one individual into small partially inbred populations. *Zoo Biology* 11:343–351.
- Spitze, K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. *Genetics* 135:367–374.

- Storfer, A., and A. Sih. 1998. Gene flow and ineffective antipredator behavior in a stream-breeding salamander. *Evolution* **52**:558-565.
- van Treuren, R., R. Bijlsma, W. van Delden, and N. J. Ouborg. 1991. The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. *Heredity* **66**:181-189.
- Wade, M. J., and C. J. Goodnight. 1991. Wright's shifting balance theory: an experimental study. *Science* **254**:1015-1018.
- Whitlock, M. C., and D. E. McCauley. 1999. Indirect measures of gene flow and migration: $F_{st} \neq 1/(4Nm+1)$. *Heredity* **83**:117-125.
- Willis, J. 1993. Effects of different levels of inbreeding on fitness components in *Mimulus guttatus*. *Evolution* **47**:864-876.
- Wright, S. 1931. Evolution in Mendelian populations. *Genetics* **16**:97-159.
- Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding, and selection in evolution. *Proceedings of the Sixth International Congress of Genetics* **1**:356-366.
- Wright, S. 1951. The genetical structure of populations. *Annals of Eugenics* **15**:323-354.
- Wright, S. 1969. *Evolution and the genetics of populations. 2. The theory of gene frequencies.* University of Chicago Press, Chicago.
- Wright, S. 1977. *Evolution and the genetics of populations. 3. Experimental results and evolutionary deductions.* University of Chicago Press, Chicago.

