American Journal of Botany 83(11): 1416–1421. 1996.

PATTERNS OF GENETIC VARIABILITY WITHIN AND AMONG POPULATIONS OF WILD RADISH, *RAPHANUS RAPHANISTRUM* (BRASSICACEAE)[1]

SUZANNE KERCHER AND JEFFREY K. CONNER[2]

Department of Ecology, Ethology, and Evolution, University of Illinois, Shelford Vivarium, 606 East Healey Street, Champaign, Illinois 61820

The genetic structure of populations is an important determinant of the evolutionary potential of a species. Colonizing plants tend to be characterized by low within- and high among-population variability. Genetic differentiation of both floral traits and isozymes was studied in six populations of wild radish (*Raphanus raphanistrum*). Evidence for differentiation in both sets of traits was found, but patterns of differentiation of floral traits did not coincide with isozyme differentiation. Contrary to most colonizing species, wild radish showed high within- and only moderate among-population variability at isozyme loci. In addition, levels of differentiation did not correspond to geographic distance between the populations. These results are likely due at least in part to the self-incompatibility system of this species, long-distance movement of large numbers of wild radish seeds by humans, and introgression from cultivated radish (*R. sativus*).

Key words: Brassicaceae; *F* statistics; genetic differentiation; genetic variability; isozymes; population genetic structure; *Raphanus raphanistrum*; wild radish.

The patterns of genetic variation within and among populations can be influenced by mutation, genetic drift, the mating system, gene flow, and selection (Slatkin, 1987). A large number of studies have measured genetic variability within and among plant populations; the majority of these studies employed isozyme markers (reviewed in Hamrick, Linhart, and Mitton, 1979; Loveless and Hamrick, 1984; Hamrick and Godt, 1989). Colonizing species usually exhibit an extreme pattern of population structure, with very low within- and high between-population variation (Hamrick, 1989). This pattern of genetic structure likely results from a variety of phenomena, including the founding of populations by a few individuals, low levels of repeated migration due to geographic isolation, novel selection in new habitats, and a propensity to self-fertilize (Barrett and Shore, 1989).

Raphanus raphanistrum (Brassicaceae) is an annual colonizing weed of agricultural fields and waste places. It originated in Europe but is now present on six continents, and was introduced in North America from Europe at least 150 yr ago (based on herbarium specimens; Panetsos and Baker, 1967; J. Conner, unpublished data). *R. raphanistrum* differs from most colonizing species (reviewed in Brown and Marshall, 1981) in two ways: it is self-incompatible (Sampson, 1964) and insect pollinated.

R. raphanistrum and the closely related *R. sativus* have become model systems for ecological and evolutionary studies (e.g., Ellstrand and Marshall, 1985b; Marshall and Ellstrand, 1986; Stanton, Snow, and Handel, 1986; Mazer, 1987; Snow and Mazer, 1988; Devlin, Clegg, and Ellstrand, 1992), but the population genetic structure of these species remains largely unstudied (but see Ellstrand and Marshall, 1985a). In this paper we examine differentiation among six natural populations of *R. raphanistrum* from the northeastern United States. Our objectives in this study were to add to the general knowledge of plant population genetic structure, especially of colonizing species, to assess *R. raphanistrum* in light of its atypical characteristics as a colonizing species, and to fill a gap in our knowledge of this widely used model organism.

MATERIALS AND METHODS

Study sites—The specimens studied were grown in a greenhouse from seeds collected at six sites (Table 1). The New York, Michigan, and Connecticut seeds were collected at regular intervals along one or more transects across each population. In Maine, populations A and B were a few kilometers apart and situated just above the mean high tide line. Population C was located ≈ 10 km inland and ≈ 50 km from Populations A and B. All individuals with ripe fruits were sampled from the three Maine populations.

Floral measurements—We measured six floral traits on one flower from each of 35—340 greenhouse-grown plants (Table 1). The Maine and Connecticut plants were measured from December 1994 to March 1995, and Michigan floral measurements were performed in June 1992 in Urbana, Illinois. New York individuals were measured in Ithaca, New York under very similar greenhouse conditions in June 1989 (see Conner and Via, 1993). Petal length, petal width, corolla tube length, long and short filament lengths, and pistil length were measured on the third to 20th flower to open on the main stem of each plant (for details, see Conner and Via, 1993). In addition, flower color was recorded for each plant; floral color in *R. raphanistrum* is determined by a single gene inherited in a Mendelian fashion, with white dominant to yellow (Stanton et al., 1989).

Since the populations were grown at three different times, there were some uncontrolled environmental differences that could have affected

[1] Manuscript received 6 September 1995; revision accepted 6 June 1996.

The authors thank A. Snow, M. Stanton, and D. Stein for generously supplying seeds, S. Berlocher for assistance with gel recipes and data analysis, T. Holtsford for a copy of his *F* statistics program, and S. Tonsor and an anonymous reviewer for comments on an earlier version of the manuscript. This research was supported by the National Science Foundation under Grant No. DEB 93–18388.

[2] Author for correspondence, current address: Kellogg Biological Station, Michigan State University, 3700 E. Gull Lake Drive, Hickory Corners, MI 49060.

TABLE 1. The six populations studied. ME = Maine, CT = Connecticut; MI = Michigan; NY = New York.

Pop.	Site	Collection year	Description	Pop. size (est.)	N (floral traits)	N (isozymes)
MEA	Ocean Pt.	1992	cobbly beach	100–200	80	84
MEB	Ocean Pt.	1992	cobbly beach	100–200	35	37
MEC	Warren	1992	pumpkin field	200–400	82	126
CT	Hamden	1984	agricultural field	≫1000	80	147
MI	Bay City	1991	sugar beet field	300–400	119	180
NY	Binghamton	1988	alfalfa field	≫1000	340	176

the floral traits. However, the greenhouse conditions we controlled such as artificial light cycle, temperature, and fertilizer were the same for each population; therefore, differences in floral measurements are likely to be primarily due to genetic differentiation for floral genes. This is certainly true for the three Maine and the Connecticut populations that were raised simultaneously. In general, floral traits are less affected by the environment than vegetative traits (Bradshaw, 1965; Conner and Via, 1993).

Electrophoresis—Unopened flower buds were collected from 37 to 180 plants in each population (Table 1) and stored in an ultracold freezer (−74°C) until electrophoresis was performed. Standard starch gel electrophoresis procedures were followed (Murphy et al., 1990; for details, see Conner et al., in press). Based upon earlier trials with the New York *R. raphanistrum* as well as family studies, we determined that eight loci were easily interpretable, inherited in a Mendelian fashion, and were not tightly linked (Conner et al., in press). The loci were: formate dehydrogenase (FDH; EC 1.2.1.2; dimer), isocitrate dehydrogenase (IDH; EC 1.1.1.42; dimer), leucine aminopeptidase (LAP; EC 3.4.11.1; monomer), phosphoglucomutase (three loci; PGM; EC 2.7.5.1; monomer), phosphoglucose isomerase (PGI; EC 5.3.1.9; dimer), and triosephosphate isomerase (TPI; EC 5.3.1.1; dimer). To ensure consistent scoring across all populations, individuals from more than one population were included on most gels.

Data analysis—Floral measurements were analyzed using SAS (SAS, 1985) and JMP (SAS, 1994). A multivariate analysis of variance (MANOVA) was performed to test for overall differences in floral morphology among the populations. Flower size was calculated for each individual as the geometric mean of the six floral traits (Mosimann and James, 1979). Pearson's correlation coefficients were also calculated for all possible pairs of floral traits within each population. The average absolute difference between correlation matrices (Willis, Coyne, and Kirkpatrick, 1991) was calculated for all possible pairs of populations.

Isozyme data were analyzed using Biosys-1, 1.7 (Swofford and Selander, 1981). Three measures of genetic variability within populations were calculated: the mean number of alleles per locus, the percentage of loci that were polymorphic (P), and an unbiased estimate of expected heterozygosity (H; Nei, 1978). We used chi-square tests to check for deviations from Hardy-Weinberg equilibrium. Nei's genetic identity and genetic distance (Nei, 1978) were used to determine relationships among the six populations, and from this information a cluster diagram was constructed. F statistics (Wright, 1978) were calculated using the methods of Weir and Cockerham (Weir and Cockerham, 1984; Weir, 1990.

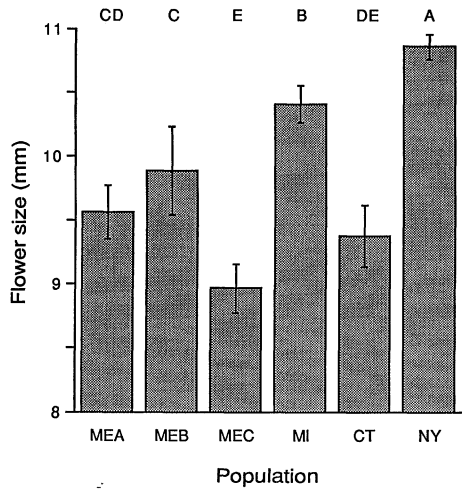

Fig. 1. Mean flower size ± 2 SE for each population. Populations that share the same letter do not differ significantly by a Tukey's test. Flower size equals the geometric mean of the six floral traits measured. Flower size differed significantly across the six populations (ANOVA: $F = 88.35$; $P < 0.0001$).

RESULTS

Floral traits—The MANOVA test for overall differences among the populations for the six floral traits was highly significant (Wilks' Lambda = 0.27, $F = 37.04$, $P < 0.0001$). Flower size also differed significantly across the populations (Fig. 1). Average absolute differences among populations in floral correlations were small, ranging from 0.02 to 0.25 with an average of 0.13 (Table 2). These results indicate that despite relatively large differences in flower size, correlation patterns were very similar across all six populations. There was no correlation

TABLE 2. Genetic and morphological differentiation of population pairs, arranged form lowest to highest genetic distance. Genetic distance (Nei, 1978) was based on isozyme data, while average absolute difference in correlations and flower size difference were based on floral morphology. Note that there is little correspondence of the morphological differentiation measures with genetic distance.

Population pair	Flower size difference	Avg. abs. diff. in correlations	Genetic distance	Gentic identity
MEC/NY	1.89	0.13	0.02	0.98
MEA/MEC	0.60	0.10	0.06	0.94
MEA/NY	1.29	0.02	0.07	0.93
MEB/MEC	0.92	0.12	0.08	0.92
MEB/NY	0.98	0.13	0.11	0.89
MEB/CT	0.50	0.08	0.14	0.87
MEC/MI	1.44	0.17	0.13	0.88
NY/MI	0.46	0.08	0.13	0.88
MEA/MEB	0.32	0.13	0.14	0.87
MEC/CT	0.41	0.08	0.16	0.85
CT/NY	1.48	0.18	0.18	0.84
CT/MI	1.03	0.25	0.19	0.83
MEA/MI	0.84	0.11	0.23	0.80
MEB/MI	0.52	0.16	0.23	0.80
MEA/CT	0.19	0.17	0.34	0.71

TABLE 3. *P* values from chi-square tests for Hardy–Weinberg equilibrium, at each locus and population separately. *P* values shown are uncorrected for multiple tests. Only individual *P* values that are <0.0005 are significant at the 0.05 level after a sequential Bonferroni correction (Rice, 1989). In addition, for each population the mean number of alleles per locus (mean alleles), percentage polymorphic loci (P; no criterion), and mean heterozygosity (*H*; unbiased estimate) is given. Standard errors are in parentheses.

Locus	MEA	MEB	MEC	MI	CT	NY
FDH	fixed	fixed	0.028	0.36	0.30	0.88
IDH	fixed	0.47	0.96	0.80	0.51	0.90
LAP	<0.0005	<0.0005	<0.0005	0.57	<0.0005	<0.0005
PGI	0.65	0.83	0.39	0.54	0.60	0.11
PGM1	<0.0005	0.003	0.45	<0.0005	<0.0005	0.94
PGM2	0.032	0.63	0.18	0.10	0.59	0.34
PGM3	0.78	0.99	0.54	0.14	0.11	0.97
TPI	0.09	0.52	0.05	0.006	0.14	0.13
Mean no. alleles	2.50 (0.38)	2.63 (0.42)	3.63 (0.38)	3.50 (0.19)	3.38 (0.26)	3.50 (0.42)
P	75%	87.5%	100%	100%	100%	100%
H	0.27 (0.10)	0.36 (0.06)	0.45 (0.07)	0.58 (0.04)	0.49 (0.06)	0.47 (0.06)

between flower size differences and correlation differences across the population pairs in Table 2 ($r = 0.18$, $P = 0.52$).

The three Maine populations and the New York population were fixed for the yellow floral morph (sample sizes in Table 1). Michigan and Connecticut also contained the white morph at frequencies of 0.49 and 0.36, respectively. In Michigan, 6% of plants had pink or bronze flowers, possibly indicating introgression of anthocyanin flower pigment genes from *R. sativus* (Panetsos and Baker, 1967).

Isozymes—Maine A and Maine B had the lowest within-population isozyme variability of the six populations, as measured by the mean number of alleles per locus, the percentage of loci polymorphic (P), and the mean heterozygosity (*H*) (Table 3). FDH, IDH, PGI, PGM-2, PGM-3, and TPI were generally in Hardy-Weinberg proportions (Table 3); no deviations at these loci were significant after a sequential Bonferroni correction (Rice, 1989), and F_{IS} values were close to zero except for TPI (Table 4).

PGM-1 and LAP did deviate significantly from Hardy–Weinberg expectations in several populations (Table 3), in all cases due to an excess of homozygotes (positive F_{IS}; Table 4). However, the presence of null alleles at both loci rather than inbreeding is the likely cause of these deviations. Null heterozygotes appear as homozygotes, usually with little or no discernible difference in banding intensity (Weeden and Wendel, 1989; S. Kercher, personal observation, University of Illinois). The presence of the LAP null allele in the New York population has been confirmed by family studies (Conner et al., in press). In addition, from another study we had isozyme data from nearly 5000 offspring of the same New York plants (Conner et al., 1996). From these offspring genotypes, we were able to determine which New York plants were heterozygotes for the LAP null allele. When we repeated the chi-square test for Hardy–Weinberg equilibrium using the recoded null allele heterozygotes, the significant deviation at the LAP locus in New York disappeared ($P = 0.33$). Based on the New York data we are confident that a null allele also existed in Maine B and Maine C, where null homozygotes (blank lanes) were scored at only the LAP locus. Maine A and Connecticut probably also harbored a hidden null allele, since the chi-square tests indicated a highly significant excess of homozygotes, which remained significant even after a Bonferroni correction; however, no null homozygotes were found. Michigan is the only population that apparently lacks a LAP null allele. Of the 190 Michigan samples run, there were no null homozygotes, and the chi-square test for Hardy–Weinberg equilibrium did not indicate any excess of homozygotes (Table 3).

Null alleles were also present at PGM-1 in at least two populations, Michigan and Connecticut. Null homozygotes were scored in both populations, and the deviations from Hardy–Weinberg expectations were due to an excess of homozygotes. Data from eight Michigan families confirm the presence of the null, and that it is inherited in a Mendelian fashion (S. Kercher, unpublished data). A null allele for PGM-1 may also exist in Maine A and Maine B. Maine B had a significant excess of homozygotes, and the chi-square was spread relatively evenly among each of three cells. The high chi-square for Maine A, however, is largely attributable to a single rare homozygote, and when cells with expected frequencies of less than five were pooled, the significance disappeared.

A trend toward excess heterozygotes was seen at TPI (negative F_{IS}; Table 4). *P* values from the chi-square tests for deviation from Hardy–Weinberg equilibrium were relatively low, but none were significant after a sequential Bonferroni correction (Table 3). This trend toward excess heterozygotes at TPI may have been due to a heterozygote advantage (reviewed in Mitton, 1989) or scoring errors caused by the faint bands this locus produced.

TABLE 4. Summary of *F* statistics (Wright, 1978) at the eight loci. Weighted mean and standard error (jackknifed over all loci) are shown.

Locus	F_{IS}	F_{ST}	F_{IT}
FDH	0.04	0.10	0.14
IDH	0.00	0.31	0.31
LAP	0.24	0.14	0.35
PGI	−0.06	0.09	0.04
PGM-1	0.25	0.15	0.37
PGM-2	0.08	0.17	0.24
PGM-3	0.01	0.10	0.11
TPI	−0.17	0.03	−0.14
Mean (SE)	0.06 (0.02)	0.14 (0.01)	0.19 (0.05)

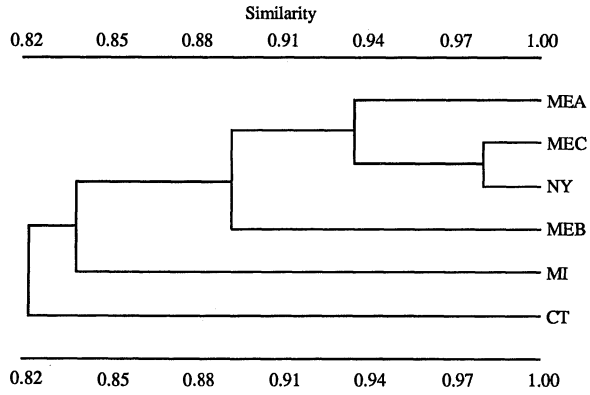

Fig. 2. Cluster diagram showing the relationships between the six populations based on Nei's (1978) genetic identity from isozyme data. ME=Maine, NY=New York, MI=Michigan, and CT=Connecticut.

F_{ST} values ranged from 0.03 for TPI to 0.31 for IDH (Table 4). Using Wright's (1978) criteria for a qualitative assessment of population differentiation, TPI differs little, IDH differs greatly, and all other loci differ moderately across the six populations. The mean F_{ST} value for all loci was 0.14, indicating moderate overall genetic differentiation among the six populations. The high F_{ST} value for IDH suggests possible diversifying selection at that locus, and the low F_{ST} value for TPI suggests that selection might favor similar genotypes across all sampled *R. raphanistrum* populations.

Nei's (1978) genetic identity was highest between New York and Maine C at 0.98, and was lowest between Connecticut and Maine A at 0.71 (Table 2). The cluster analysis indicates that New York, Maine C, and Maine A were most similar with genetic identities > 0.93 (Fig. 2). Maine B was closest to this group with a similarity of $\approx$ 0.89, while Connecticut and Michigan were distantly related to the other populations and to each other with similarities < 0.85 (Fig. 2). To determine whether the groupings may have been affected by the null alleles at LAP and PGM-1, we reran the analysis with these loci excluded. The resulting cluster diagram revealed the same relationships among the six populations as well as a very similar average F_{ST} value.

The patterns of differentiation revealed by isozymes were different from those revealed by the two measures of floral differentiation (Table 2). Genetic distance and average absolute difference in correlations were not significantly correlated ($r = 0.41$, $P = 0.13$), while genetic distance and flower size were negatively correlated with marginal significance ($r = -0.51$, $P = 0.05$).

DISCUSSION

This study provided evidence for genetic differentiation in both flower size and isozymes among the six populations. Some of the flower size differences may be attributable to uncontrolled environmental differences because plants were raised at three different times. The three Maine populations and the Connecticut population, however, were raised at the same time and still showed definite flower size differences (Fig. 1). It should be noted that even this differentiation could be due to differences in seed size or quality caused by the original field environment, but these maternal effects should be more prevalent in early plant development and most likely have less effect on floral size. The close similarity of floral correlation patterns across the six populations (low average absolute differences, Table 2) suggests a close developmental link among the six floral traits we measured and/or selection by pollinators to maintain these floral correlations (cf. Conner and Via, 1993; Conner and Sterling, 1995).

Our results showed little correspondence between patterns of genetic differentiation based on isozyme vs. morphometric data. Only half of the studies reviewed by Hamrick (1989) that compared isozyme and morphometric differentiation, however, found positive associations between the two. The disparity we found between genetic and morphological measures could indicate that selection has acted differently on isozymes and floral traits (cf. Podolsky and Holtsford, 1995), or that the floral differentiation may be due to environmental differences (see above).

The genetic structure revealed by isozymes alone did not fit general patterns for colonizing plants in several ways. First, within-population variation as measured by heterozygosity, percentage loci polymorphic, and the mean number of alleles per locus (Table 3) was above the average for most plant species (reviewed in Hamrick, 1989) and extremely high for a colonizing species, since weed populations often arise from a small number of founders and commonly self-fertilize (reviewed in Barrett and Shore, 1989). Wild radish may not fit either of these characteristics, however. The self-incompatibility mechanism not only prevents selfing, but also reduces the frequency of matings between close relatives. Since outcrossing weeds are rare, few reports of genetic variability exist (Barrett, 1982), but the levels of heterozygosity reported here are still generally higher than that reported for six other outcrossing weeds ($H = 0.11$—0.45; Brown and Burdon, 1983; Ellstrand and Marshall, 1985a; Warwick, Thompson, and Black, 1987; Rieseberg, Soltis, and Soltis, 1988; Van Dijk, Wolff, and De Vries, 1988; Simonsen and Heneen, 1995).

In addition, long-distance movement of large numbers of wild radish seeds by humans occurs through contamination of small grain seeds such as wheat, oats, barley, and rye; this dispersal mechanism may have been quite common in the past and still occurs today (G. Amberson, personal communication, University of Wisconsin; S. Strauss, personal communication, University of California). Even if a single plant were accidentally collected with grain seed, potentially hundreds of individual seeds could be carried to a new field with optimal disturbed conditions for establishing a new population. In this respect, it is interesting that the two coastal Maine populations, perhaps representative of a more natural method of long-distance seed dispersal via water, had lower levels of heterozygosity and lower mean numbers of alleles per locus than the four populations located in agricultural fields. Thus a strong determinant of population structure of some agricultural weeds like *Raphanus raphanistrum* might be the prevalence of populations established by an unnaturally high number of founders through long-distance dispersal by humans. Indeed, movement of rela-

tively large groups of seeds may be required for the establishment of self-incompatible weeds.

The second unusual result of our study was the moderate levels of between-population differentiation. Colonizing plants generally exhibit high between-population genetic variation; our average F_{ST} value of 0.14 (Table 4) was similar to an average of 0.16 for 15 outcrossed annuals, but much lower than average compared to other insect-pollinated (average F_{ST} = 0.23) or early-successional (average F_{ST} = 0.41) species (Loveless and Hamrick, 1984). Our results were not very different from studies of five other self-incompatible weeds, which found F_{ST} values ranging from 0.01 to 0.22 (Ellstrand and Marshall, 1985a; Warwick, Thompson, and Black, 1987; Rieseberg, Soltis, and Soltis, 1988; Van Dijk, Wolff, and De Vries, 1988; Simonsen and Heneen, 1995). Again, human and possibly water transport of many seeds at a time over long distances are probably the main causes of the relatively low variation among populations in wild radish and possibly some other outcrossing weeds; differentiation is reduced when the number of individuals founding a population is large (Wade and McCauley, 1988).

The final unusual result was that the pattern of isozyme differentiation among the six populations did not fit that predicted by simple isolation by distance. The Maine C and New York populations, which were > 400 km apart, had a higher genetic identity than Maine A and Maine B, which were only a few kilometres apart. Once again, long-range movement of seeds by humans probably accounts for much of this pattern.

Another possible cause for the lack of geographical pattern of differentiation is introgression of genes from *R. sativus* (garden radish). The pink and bronze flowers in the Michigan population are evidence for the occurrence of this introgression. Crops and their wild, weedy relatives often lack reproductive barriers and are often found sympatrically (Doebley, 1989). Evidence for introgression into crop plants from wild relatives and, less frequently, vice versa, has been documented for a number of species including *Lycopersicon* (Rick, Zobel, and Fobes, 1974; Rick and Fobes, 1975), *Zea* (Doebley, 1984; Doebley, Goodman, and Stuber, 1987), and *Cucurbita* (Kirkpatrick and Wilson, 1988). Klinger, Elam, and Ellstrand (1991) experimentally manipulated the distance between populations of cultivated and wild *R. sativus* to determine rates of introgression, and they found that gene flow still occurred substantially between the two at distances up to 1 km. Introgression from *R. sativus* into *R. raphanistrum* is indeed a possibility as well; greenhouse crosses have demonstrated that the two species are fully compatible (A. Snow, personal communication, Ohio State University; Panetsos and Baker, 1967).

Finally, while it might be expected that the introduction of *R. raphanistrum* to North America would have caused a reduction in within-population diversity through a combination of population bottlenecks, an absence of repeated migration, and novel selection pressures (Barrett and Shore, 1989), a small study of one British *R. raphanistrum* population actually reported a much lower percentage polymorphic loci and heterozygosity than we found (Lewis-Jones, Thorpe, and Wallis, 1982). Clearly, only a more comprehensive study of *R. raphanistrum* populations, perhaps focusing more on natural populations where human dispersal was an unlikely source and perhaps including representatives from all six continents where it is now found, would reveal whether the patterns we found are truly typical for this species.

LITERATURE CITED

BARRETT, S. C. H. 1982. Genetic variation in weeds. *In* R. Charudattan and H. L. Walker [eds.], Biological control of weeds with plant pathogens, 73–98. John Wiley & Sons, New York, NY.

———, AND J. S. SHORE. 1989. Isozyme variation in colonizing plants. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 127–145. Dioscorides Press, Portland, OR.

BRADSHAW, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* 13: 115–155.

BROWN, A. H. D., AND J. J. BURDON. 1983. Miltilocus diversity in an outbreeding weed, *Echium plantagineum* L. *Australian Journal of Biological Sciences* 36: 503–509.

BROWN, A. H. D., AND D. R. MARSHALL. 1981. Evolutionary changes accompanying colonization in plants. *In* G. C. E. Scudder and J. L. Reveal [eds.], Evolution today, 351–363. Hunt Institute for Botanical Documentation, Carnegie-Mellon University, Pittsburgh, PA.

CONNER, J. K., AND S. VIA. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution* 47: 704–711.

———, AND A. STERLING. 1995. Testing hypotheses of functional relationships: a comparative survey of correlation patterns among floral and vegetative traits in five insect-pollinated plants. *American Journal of Botany* 82: 1399–1406.

———, S. RUSH, S. KERCHER, AND P. JENNETTEN. 1996. Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). II. Selection through lifetime male and total fitness. *Evolution* 50: 1137–1146.

———, D. TJHIO, S. H. BERLOCHER, AND S. L. RUSH. In press. Inheritance and linkage relationships of nine isozyme loci in wild radish. *Journal of Heredity*.

DEVLIN, B., J. CLEGG, AND N. C. ELLSTRAND. 1992. The effect of flower production on male reproductive success in wild radish populations. *Evolution* 46: 1030–1042.

DOEBLEY, J. F. 1984. Maize introgression into teosinte—a reappraisal. *Annals of the Missouri Botanical Garden* 71: 1100–1113.

———. 1989. Isozymic evidence and the evolution of crop plants. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 127–145. Dioscorides Press, Portland, OR.

———, M. M. GOODMAN, AND C. W. STUBER. 1987. Patterns of isozyme variation between maize and Mexican annual teosinte. *Economic Botany* 41: 234–246.

ELLSTRAND, N. C., AND D. L. MARSHALL. 1985a. The impact of domestication on distribution of allozyme variation within and among cultivars of radish, *Raphanus sativus* L. *Theoretical and Applied Genetics* 69: 393–398.

———, AND ———. 1985b. Interpopulation gene flow by pollen in wild radish, *Raphanus sativus*. *American Naturalist* 126: 606–616.

HAMRICK, J. L. 1989. Isozymes and the analysis of genetic structure in plant populations. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 87–105. Dioscorides Press, Portland, OR.

———, Y. B. LINHART, AND J. B. MITTON. 1979. Relationships between life history characteristics and electrophoretically-detectable genetic variation in plants. *Annual Review of Ecology and Systematics* 10: 173–200.

———, AND M. J. W. GODT. 1989. Allozyme diversity in plant species. *In* A. H. D. Brown et al. [ed.], Plant population genetics, breeding, and genetic resources, 43–63. Sinauer, Sunderland, MA.

KIRKPATRICK, K. J., AND H. D. WILSON. 1988. Interspecific gene flow in *Cucurbita*: *C. texana* vs. *C. pepo*. *American Journal of Botany* 75: 519–527.

KLINGER, T., D. R. ELAM, AND N. C. ELLSTRAND. 1991. Radish as a model system for the study of engineered gene escape rates via crop-weed mating. *Conservation Biology* 5: 531–535.

LEWIS-JONES, L. J., J. P. THORPE, AND G. P. WALLIS. 1982. Genetic divergence in four species of the genus *Raphanus*: implications for

the ancestry of the domestic radish *R. sativus*. *Biological Journal of the Linnean Society* 18: 35–48.

LOVELESS, M. D., AND J. L. HAMRICK. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65–95.

MARSHALL, D. L., AND N. C. ELLSTRAND. 1986. Sexual selection in *Raphanus sativus*: experimental data on nonrandom fertilization, maternal choice, and consequences of multiple paternity. *American Naturalist* 127: 446–461.

MAZER, S. J. 1987. The quantitative genetics of life history and fitness components in *Raphanus raphanistrum* L. (Brassicaceae): ecological and evolutionary consequences of seed-weight variation. *American Naturalist* 130: 891–914.

MITTON, J. B. 1989. Physiological and demographic variation associated with allozyme variation. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 127–145. Dioscorides Press, Portland, OR.

MOSIMANN, J. E., AND F. C. JAMES. 1979. New statistical methods for allometry with application to Florida red-winged blackbirds. *Evolution* 33: 444–459.

MURPHY, R. W., J. W. SITES JR., D. G. BUTH, AND C. H. HAUFLER. 1990. Proteins. I. Isozyme electrophoresis. *In* D. M. Hillis and C. Moritz [eds.], Molecular systematics, 45–126. Sinauer, Sunderland, MA.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 86: 187–198.

PANETSOS, C. A., AND H. G. BAKER. 1967. The origin of variation in "wild" *Raphanus sativus* (Cruciferae) in California. *Genetics* 38: 243–274.

PODOLSKY, R. H., AND T. P. HOLTSFORD. 1995. Population structure of morphological traits in *Clarkia dudleyana*. I. Comparison of F_{st} between allozymes and morphological traits. *Genetics* 140: 733–744.

RICE, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223–225.

RICK, C. M., R. W. ZOBEL, AND J. F. FOBES. 1974. Four peroxidase loci in red-fruited tomato species: genetics and geographic distribution. *Proceedings of the National Academy of Sciences, USA* 71: 835–839.

———, AND J. F. FOBES. 1975. Allozyme variation in the cultivated tomato and closely related species. *Bulletin of the Torrey Botanical Club* 102: 376–384.

RIESEBERG, L. H., D. E. SOLTIS, AND P. S. SOLTIS. 1988. Genetic variation in *Helianthus annuus* and *H. bolanderi*. *Biochemical Systematics and Ecology* 16: 393–399.

SAMPSON, D. R. 1964. A one-locus self-incompatibility system in *Raphanus raphanistrum*. *Canadian Journal of Genetics and Cytology* 6: 435–445.

SAS. 1985. SAS user's guide: Statistics. SAS Institute, Cary, NC.

———. 1994. JMP, ver. 3. SAS Institute, Cary, NC.

SIMONSEN, V., AND W. K. HENEEN. 1995. Genetic variation within and among different cultivars and landraces of *Brassica campestris* L. and *B. oleracea* L. based on isozymes. *Theoretical and Applied Genetics* 91: 346–352.

SLATKIN, M. 1987. Gene flow and the geographic structure of populations. *Science* 236: 787–792.

SNOW, A., AND S. J. MAZER. 1988. Gametophytic selection in *Raphanus raphanistrum*: a test for heritable variation in pollen competitive ability. *Evolution* 42: 1065–1075.

STANTON, M. L., A. A. SNOW, AND S. N. HANDEL. 1986. Floral evolution: attractiveness to pollinators increases male fitness. *Science* 232: 1625–1627.

———, ———, ———, AND J. BERECZKY. 1989. The impact of a flower-color polymorphism on mating patterns in experimental populations of wild radish (*Raphanus raphanistrum* L.). *Evolution* 43: 335–346.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* 72: 281–283.

VAN DIJK, H., K. WOLFF, AND A. DE VRIES. 1988. Genetic variability in *Plantago* species in relation to their ecology. 3. Genetic structure of populations of *P. major, P. lanceolata* and *P. coronopus*. *Theoretical and Applied Genetics* 75: 518–528.

WADE, M. J., AND D. E. MCCAULEY. 1988. Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution* 42: 995–1005.

WARWICK, S. I., B. K. THOMPSON, AND L. D. BLACK. 1987. Genetic variation in Canadian and European populations of the colonizing weed species *Apera spica-venti*. *New Phytologist* 106: 301–317.

WEEDEN, N. F., AND J. F. WENDEL. 1989. Genetics of plant isozymes. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 127–145. Dioscorides Press, Portland, OR.

WEIR, B. S. 1990. Genetic data analysis. Sinauer, Sunderland, MA.

———, AND C. C. COCKERHAM. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.

WILLIS, J. H., J. A. COYNE, AND M. KIRKPATRICK. 1991. Can one predict the evolution of quantitative characters without genetics? *Evolution* 45: 441–444.

WRIGHT, S. 1978. Evolution and the genetics of populations, vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago, IL.