

Comparison of pollinator flight movements and gene dispersal patterns in *Mimulus ringens*

JEFFREY D. KARRON*, ROSELLA TUCKER, NINA N. THUMSER & JAMES A. REINARTZ†

Department of Biological Sciences and †Field Station, PO Box 413, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, U.S.A.

Pollinator movements and pollen-mediated gene dispersal were quantified in experimental populations of square-stemmed monkeyflower (*Mimulus ringens*), a wetland perennial herb with a mixed-mating system. Each population consisted of genets with unique multilocus combinations of homozygous genotypes, facilitating assignment of paternity to all sampled seeds. Replicate arrays were planted at each of three spacings spanning the range of densities typically observed in natural *M. ringens* populations. In all six arrays the distribution of gene dispersal distances differed significantly from the distribution of pollinator flight movements. The mean gene dispersal distance was 1.46 times as far as the mean pollinator flight distance. These differences were probably caused by pollen carryover as bumblebees visited up to 12 *M. ringens* plants on a single foraging trip. Although gene movements exceeded pollinator flight distances, estimates of neighbourhood size were consistently low, ranging from 1.66 to 5.53. Therefore, high levels of random local genetic differentiation are likely in this species.

Keywords: gene dispersal, *Mimulus ringens*, neighbourhood size, paternity analysis, pollinator movements, pollen carryover.

Introduction

Pollen-mediated gene movements influence genetic structure (Wright, 1931, 1938; Jain & Bradshaw, 1966; Turner *et al.*, 1982) and neighbourhood size (Wright, 1946; Levin & Kerster, 1974; Crawford, 1984a,b) in zoophilous plant populations. Pollinators frequently move short distances (Bateman, 1947b; Levin & Kerster, 1969a,b; Fenster, 1991), potentially limiting gene dispersal. However, pollinator flight data may underestimate the extent of gene movement within populations because of the effects of pollen carryover (Price & Waser, 1979; Schaal, 1980; Thomson & Plowright, 1980; Thomson & Thomson, 1989) and post-pollination events influencing fertilization (Campbell, 1991; Marshall & Folsom, 1991; Krauss, 1994). Several researchers have quantified patterns of gene dispersal by introducing one or two marker genotypes into populations lacking the novel alleles (Bateman, 1947a; Schaal, 1980; Levin, 1981; Ennos & Clegg, 1982; Handel, 1982; Galen, 1992).

This approach provides valuable information about patterns of gene dispersal from the marker plants, but does not permit measurement of gene movement from other plants in the population.

Paternity exclusion analysis enhances description of plant mating patterns (Ellstrand, 1984; Hamrick & Schnabel, 1985; Meagher, 1986; Devlin & Ellstrand, 1990; Broyles & Wyatt, 1991; Godt & Hamrick, 1993). Studies that assign a unique paternal parent to each sampled seed (Broyles & Wyatt, 1991) provide an especially detailed description of gene movements. In this paper we characterize patterns of gene dispersal within replicate experimental populations of *Mimulus ringens* (square-stemmed monkeyflower). To facilitate assignment of paternity to every sampled seed, each population consisted of genets with unique multilocus combinations of homozygous genotypes (Karron *et al.*, 1995, Karron, 1996). We test the hypothesis that patterns of gene dispersal within populations will differ from patterns of pollinator movement because of the effects of pollen carryover. We also estimate neighbourhood size at a range of population densities.

*Correspondence.

Materials and methods

Study organism

Mimulus ringens L. (Scrophulariaceae) is a diploid perennial herb that is broadly distributed in wetlands of central and eastern North America (Grant, 1924). Populations tend to be small (less than 50 individuals) and occur at a wide range of densities. Plants flower in July and August and are pollinated by workers and queens in the genus *Bombus* (bumblebees). Like many of its congeners (Sutherland & Vickery, 1988; Ritland & Ritland, 1989; Dudash & Ritland, 1991; Dole, 1992), *M. ringens* is self-compatible and has a mixed-mating system. Outcrossing rates range from 0.15 to 0.33 and are positively correlated with population density (Karron *et al.*, 1995).

Establishment of experimental populations

The breeding programme for production of the marker genotypes is described in detail in Karron *et al.* (1995). Key features of the experimental design are as follows. Each of the 16 plants in a population had a unique combination of homozygous genotypes at four allozyme loci: acid phosphatase (*Acp-1*, EC 3.1.3.2), aconitase (*Aco-3*, EC 4.2.1.3), glutamic-oxalacetic transaminase (*Got-1*, EC 2.6.1.1) and shikimate dehydrogenase (*Skd-1*, EC 1.1.1.25). These marker genotypes were produced by crossing multilocus heterozygotes derived from a single natural population. Plants for six populations were then clonally propagated from the original set of marker genotypes to minimize differences in floral and vegetative morphology among populations.

Each replicate set of genets was planted in a separate garden at the UW-Milwaukee Field Station (Saukville, WI). The gardens were tilled prior to planting and mulched with hay to control weeds. To minimize among-population gene flow, which can not be detected with our experimental design, each garden was separated by 75 m of old field vegetation containing a high density of bumblebee-pollinated flowers. Long-distance gene flow was unlikely as the nearest natural population of *M. ringens* was more than 15 km away.

Replicate arrays were planted at each of three spacings which span the range of densities typically observed in natural populations of *M. ringens*. Two arrays were planted at 'high density' (0.6 m spacing between plants), two were planted at 'medium density' (1.2 m spacing) and two were planted at 'low density' (2.4 m spacing). Each array had four parallel rows, with four evenly-spaced plants per

row. Positions of clones in each population were randomly assigned.

Pollinator observations

The six arrays had similar numbers of flowers and comparable rates of floral visitation by pollinators (Karron *et al.*, 1995). Patterns of among-plant pollinator movement were noted on 8 days during the peak period of flowering. Each day, sampling intervals of 20 min were assigned in random order to the six populations. During an observation period, the exact sequence of plants visited by every pollinator was recorded. A PASCAL program was written to calculate the absolute and relative distances of all flight segments from matrices of the row and column positions of the 16 genets in each array. The program (available from J. Reinartz on request) assigns a relative distance of 1 to flights between nearest neighbours, ($\sqrt{2} = 1.4$) to flights between diagonal neighbours and values of 2–4.2 to more distant pollinator movements.

Assignment of paternity

Thirty-five fruits were harvested from each maternal plant in the six experimental arrays. All seeds sampled from an individual were pooled together and a portion was sown in a single flat. Germination rates exceeded 90 per cent. Twenty 6-week-old seedlings were randomly selected from each of the 96 maternal families and these 1920 seedlings were genotyped at the four allozyme loci. Electrophoretic methods are described in Karron *et al.* (1995).

As maternal plants had unique combinations of homozygous genotypes at four loci, progeny resulting from selfing and progeny produced by outcrossing could readily be distinguished (Karron *et al.*, 1995). The paternity of all outcross progeny was determined with a simple exclusion procedure (Ellstrand, 1984; Brown *et al.*, 1989; Karron *et al.*, 1995, Karron, 1996). Absolute and relative distances between the maternal parent and pollen donor of each outcross seed were then calculated using the PASCAL program described above.

Data analyses

After flowering commenced, we discovered that three of the 16 clonal lines had very low levels of pollen production and reduced seed set in every array. This trait occurs in natural populations of *M. ringens* and is inherited as a single recessive gene (Karron *et al.*, 1995). Because pollinator movements

from these plants do not result in gene dispersal, we excluded data on pollinator flights and gene dispersal to and from these genets.

Among the 78 plants remaining in the analysis, we recorded 544 pollinator flight segments and 349 outcross gene movements. Each of these was assigned to one of five relative distance categories (1.0, 1.4, 2–2.2, 2.8–3.2 or 3.6–4.2) to ensure adequate sample size in cells at the tail end of the frequency distributions. Data on frequencies of pollinator and gene movements in each population were organized into contingency tables and tested with a chi-square analysis (Steel & Torrie, 1980; SAS Institute, 1990).

For each array, the variance of pollen-mediated gene dispersal (σ_p^2) was calculated from the absolute distances of gene dispersal within populations (p)

using the formula $\sigma_p^2 = \sum p^2/n_p$ (Crawford, 1984a,b). This estimate of σ_p^2 assumes no interpopulation gene flow, which could not be measured in this study because the six populations had the same set of multilocus genotypes. The neighbourhood size (N_e) was then estimated by

$$N_e = 4\pi(t\sigma_p^2/2 + \sigma_s^2)(d/2)(1+t),$$

where d is the population density, σ_s^2 is the variance of seed dispersal (not measured and treated as 0) and t is the population outcrossing rate (Crawford, 1984b). Outcrossing rate estimates for each experimental array are reported in Karron *et al.* (1995). To examine the influence of outcrossing rate on neighbourhood size, we also calculated N_e of each population using a constant $t = 0.23$ (the mean outcrossing rate in the six arrays).

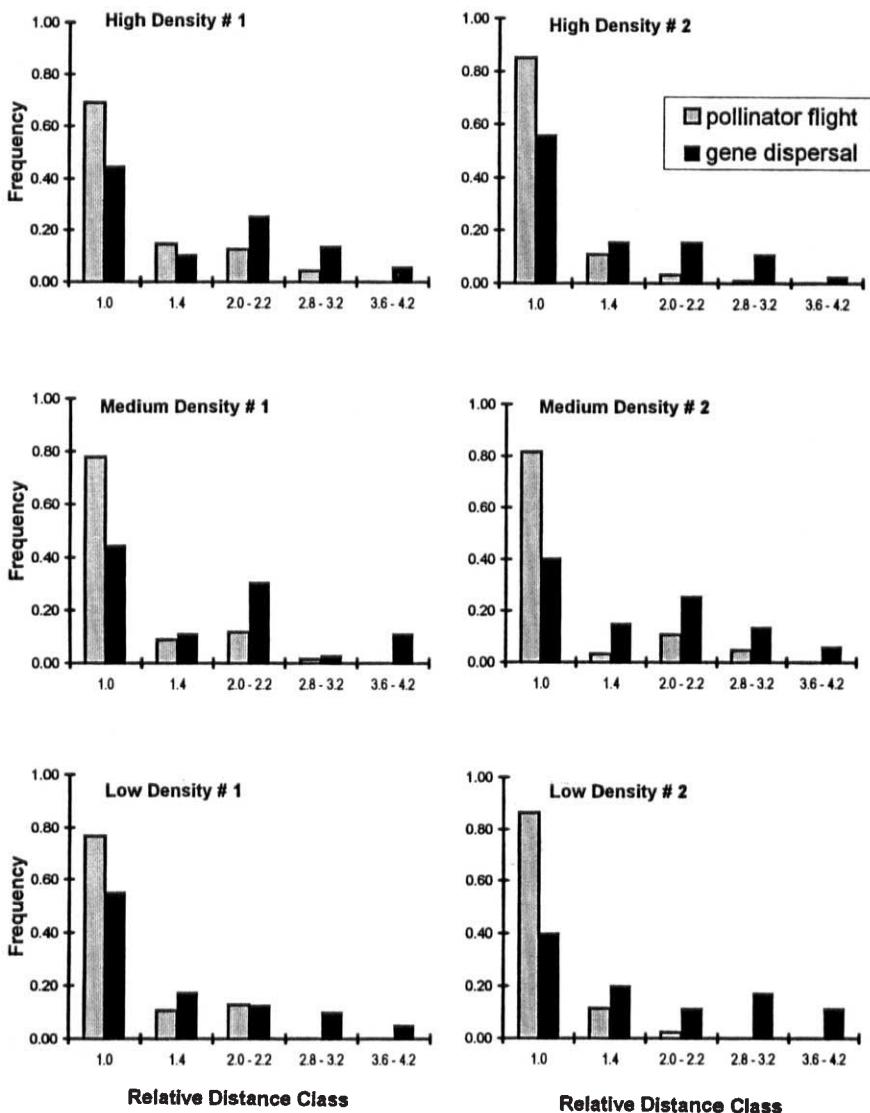


Fig. 1 Patterns of pollinator movement and gene dispersal in six experimental populations of *Mimulus ringens*. Each graph shows the proportion of pollinator flights and the proportion of gene dispersal movements in each relative distance category. See text for data analyses.

Results

Patterns of pollinator movement and gene dispersal in the six arrays are shown in Fig. 1. In each population, there were significant differences between the proportions of bumblebee flights and gene movements in the five relative distance classes: high density population 1, $\chi^2_4 = 19.9$, $P < 0.001$; high density population 2, $\chi^2_4 = 43.9$, $P < 0.0001$; medium density population 1, $\chi^2_4 = 16.4$, $P < 0.005$; medium density population 2, $\chi^2_4 = 24.9$, $P < 0.001$; low density population 1, $\chi^2_4 = 9.5$, $P < 0.05$; low density population 2, $\chi^2_4 = 22.5$, $P < 0.001$.

In all six populations, most ($\bar{X} = 79.5$ per cent) pollinator flights were between nearest neighbours, whereas fewer than half ($\bar{X} = 46.7$ per cent) of all gene movements were between adjacent mates (Fig. 1). On average, the mean distance of gene dispersal was 1.46 times as far as the mean pollinator flight distance (Table 1).

Although most bumblebee flights were between neighbouring plants, the mean number of plants

visited during a foraging trip was 4.80 ± 0.25 . Nine per cent of the pollinators visited 10–12 plants before leaving the population.

The variance in absolute distance of gene dispersal (σ_p^2) was inversely proportional to population density. Therefore, when a constant value of t was used to calculate neighbourhood size, there was no relationship between density and N_e (Table 2). Because outcrossing rates were higher in the dense populations (Karron *et al.*, 1995), estimates of N_e incorporating t for each population were positively correlated with density ($r = 0.80$, $P = 0.055$, d.f. = 4).

Discussion

In each of the *M. ringens* populations, the mean distance of gene dispersal exceeded the mean distance of pollinator movement. These differences were probably caused by pollen carryover, which occurs when pollen collected from a plant is dispersed not only to the next plant, but also to

Table 1 Ratios of the mean distance of gene dispersal divided by the mean distance of pollinator movement in six populations of *Mimulus ringens*

Population	Mean distance of gene dispersal
	Mean distance of pollinator movement
High density no. 1	1.390
High density no. 2	1.389
Medium density no. 1	1.462
Medium density no. 2	1.459
Low density no. 1	1.307
Low density no. 2	1.749
Mean for 6 populations	1.459

Values > 1 indicate that the mean distance of gene dispersal exceeded the mean distance of pollinator movement.

Table 2 Estimates of neighbourhood size (N_e) in six populations of *Mimulus ringens*

Population	n	d	σ_p^2	t	N_e (constant $t = 0.23$) ^a	N_e (t of each pop.) ^b
Low density no. 1	40	0.17	17.14	0.15	2.64	1.66
Low density no. 2	35	0.17	26.00	0.15	4.01	2.45
Medium density no. 1	36	0.69	5.44	0.15	3.36	2.05
Medium density no. 2	67	0.69	5.76	0.26	3.55	4.07
High density no. 1	87	2.78	1.42	0.33	3.50	5.53
High density no. 2	84	2.78	1.05	0.32	2.59	3.92

n is the number of outcross seeds genotyped, d is the population density (plants/m²), σ_p^2 is the variance of pollen-mediated gene dispersal, and t is the outcrossing rate.

To highlight the influence of outcrossing rate on neighbourhood size, N_e is calculated in two ways: ^a using a constant outcrossing rate of 0.23 in each population; ^b using the estimated outcrossing rate of each population.

subsequent plants visited by a pollinator (Price & Waser, 1979; Thomson & Plowright, 1980; Thomson & Thomson, 1989). As bumblebees visited up to 12 *M. ringens* plants in a single foraging trip, there was considerable opportunity for pollen carryover to occur.

The results of our study, based on paternity data, closely parallel the findings of earlier investigations utilizing one or two marker genotypes to quantify gene dispersal patterns in native plant populations. Schaal (1980) established an experimental array of *Lupinus texensis* to quantify dispersal of two rare *Pgi* alleles in a population homozygous for a third common allele. She found that the mean distance of gene dispersal was 1.88 times as far as the mean distance travelled by bee pollinators.

Levin (1981) introduced a *Phlox drummondii* cultivar with a dominant corolla lobe character into a natural population lacking the dominant allele. The mean distance of gene dispersal was 1.5 times as far as the mean distance flown by lepidopteran pollinators.

Only one previous study has utilized complete paternity analysis to compare pollinator movements with patterns of gene dispersal. Broyles & Wyatt (1991) quantified butterfly movements and realized gene dispersal in a natural population of self-incompatible *Asclepias exaltata*. The mean distance of gene dispersal was approximately three times the mean pollinator flight distance. These researchers attributed the high level of pollen carryover to the floral biology of *Asclepias*. Pollen carryover is especially likely in this genus because several flowers must usually be visited by a pollinator before a pollinium is inserted into a stigmatic chamber (Broyles & Wyatt, 1991; Nilsson *et al.*, 1992; Shore, 1993).

Although gene movements within our *M. ringens* populations exceeded bumblebee flight distances, estimates of neighbourhood size were consistently low, ranging from 1.66 to 5.53. These neighbourhood sizes should be regarded as minimum estimates as seed dispersal and among-population gene flow were not measured. Significant amounts of gene flow have been detected in outcrossing species such as *Raphanus sativus* (Ellstrand *et al.*, 1989) and *Asclepias exaltata* (Broyles *et al.*, 1994). Small amounts of gene flow between the experimental populations in this study would increase the variance in gene dispersal.

Wright (1946) noted that populations with $N_e < 20$ are likely to have a high level of random local genetic differentiation. The low N_e estimates in our study reflect low rates of outcrossing and relatively short distances of pollen-mediated gene dispersal

within populations. Although studies of seed dispersal and long-distance gene flow are needed, our data suggest that high levels of local genetic differentiation may be likely in this species.

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References

- BATEMAN, A. J. 1947a. Contamination of seed crops. I. Insect pollination. *J. Genet.*, **48**, 257–275.
- BATEMAN, A. J. 1947b. Contamination in seed crops. III. Relation with isolation distance. *Heredity*, **1**, 303–336.
- BROWN, A. H. D., BURDON, J. J. AND JAROSZ, A. M. 1989. Isozyme analysis of plant mating systems. In: Soltis, D. E. and Soltis, P. S. (eds) *Isozymes in Plant Biology*, pp. 73–86. Dioscorides Press, Portland, OR.
- BROYLES, S. B., SCHNABEL, A. AND WYATT, R. 1994. Evidence for long-distance pollen dispersal in milkweeds (*Asclepias exaltata*). *Evolution*, **48**, 1032–1040.
- BROYLES, S. B. AND WYATT, R. 1991. Effective pollen dispersal in a natural population of *Asclepias exaltata*: the influence of pollinator behavior, genetic similarity, and mating success. *Am. Nat.*, **138**, 1239–1249.
- CAMPBELL, D. R. 1991. Comparing pollen dispersal and gene flow in a natural population. *Evolution*, **45**, 1965–1968.
- CRAWFORD, T. J. 1984a. The estimation of neighbourhood parameters for plant populations. *Heredity*, **52**, 273–283.
- CRAWFORD, T. J. 1984b. What is a population? In: Shorrocks, B. (ed.) *Evolutionary Ecology*, pp. 135–173. Blackwell Scientific Publications, Oxford.
- DEVLIN, B. AND ELLSTRAND, N. C. 1990. Male and female fertility variation in wild radish, a hermaphrodite. *Am. Nat.*, **136**, 87–107.
- DOLE, J. A. 1992. Reproductive assurance mechanisms in three taxa of the *Mimulus guttatus* complex (Scrophulariaceae). *Am. J. Bot.*, **79**, 650–659.
- DUDASH, M. R. AND RITLAND, K. 1991. Multiple paternity and self-fertilization in relation to floral age in *Mimulus guttatus* (Scrophulariaceae). *Am. J. Bot.*, **78**, 1746–1753.
- ELLSTRAND, N. C. 1984. Multiple paternity within the fruits of the wild radish, *Raphanus sativus*. *Am. Nat.*, **123**, 819–828.
- ELLSTRAND, N. C., DEVLIN, B. AND MARSHALL, D. L. 1989. Gene flow by pollen into small populations: data from

- experimental and natural stands of wild radish. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 9044–9047.
- ENNOS, R. A. AND CLEGG, M. T. 1982. Effect of population substructuring on estimates of outcrossing rate in plant populations. *Heredity*, **48**, 283–292.
- FENSTER, C. B. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae). I. Gene dispersal. *Evolution*, **45**, 398–409.
- GALEN, C. 1992. Pollen dispersal dynamics in an alpine wildflower, *Polemonium viscosum*. *Evolution*, **46**, 1043–1051.
- GODT, M. J. W. AND HAMRICK, J. L. 1993. Patterns and levels of pollen-mediated gene flow in *Lathyrus latifolius*. *Evolution*, **47**, 98–110.
- GRANT, A. L. 1924. A monograph of the genus *Mimulus*. *Ann. Mo. Bot. Gard.*, **11**, 99–388.
- HAMRICK, J. L. AND SCHNABEL, A. 1985. Understanding the genetic structure of plant populations: some old problems and a new approach. In: Gregorius, H.-R. (ed.). *Lecture Notes in Biomathematics*, vol. 60: *Population Genetics in Forestry*, pp. 50–70. Springer, Berlin.
- HANDEL, S. N. 1982. Dynamics of gene flow in an experimental population of *Cucumis melo* (Cucurbitaceae). *Am. J. Bot.*, **69**, 1538–1546.
- JAIN, S. K. AND BRADSHAW, A. D. 1966. Evolutionary divergence among adjacent plant populations. I. The evidence and its theoretical analysis. *Heredity*, **21**, 407–441.
- KARRON, J. D. 1996. Genetic consequences of different patterns of distribution and abundance. In: Kunin, W. E. and Gaston, K. J. (eds) *The Biology of Rarity*. Chapman & Hall, London (in press).
- KARRON, J. D., THUMSER, N. N., TUCKER, R. AND HESSENAUER, A. J. 1995. The influence of population density on outcrossing rates in *Mimulus ringens*. *Heredity*, **75**, 175–180.
- KRAUSS, S. L. 1994. Restricted gene flow within the morphologically complex species *Persoonia mollis* (Proteaceae): contrasting evidence from the mating system and pollen dispersal. *Heredity*, **73**, 142–154.
- LEVIN, D. A. 1981. Dispersal versus gene flow in plants. *Ann. Mo. Bot. Gard.*, **68**, 232–253.
- LEVIN, D. A. AND KERSTER, H. W. 1969a. The dependence of bee-mediated pollen and gene dispersal upon plant density. *Evolution*, **23**, 560–571.
- LEVIN, D. A. AND KERSTER, H. W. 1969b. Density-dependent gene dispersal in *Liatris*. *Am. Nat.*, **103**, 61–74.
- LEVIN, D. A. AND KERSTER, H. W. 1974. Gene flow in seed plants. *Evol. Biol.*, **7**, 139–220.
- MARSHALL, D. L. AND FOLSOM, M. W. 1991. Mate choice in plants: an anatomical to population perspective. *Ann. Rev. Ecol. Syst.*, **22**, 37–63.
- MEAGHER, T. R. 1986. Analysis of paternity within a natural population of *Chamaelirium luteum*. I. Identification of most-likely male parents. *Am. Nat.*, **128**, 199–215.
- NILSSON, L. A., RABAKONANDRIANINA, E. AND PETTERSSON, B. 1992. Exact tracking of pollen transfer and mating in plants. *Nature*, **360**, 666–668.
- PRICE, M. V. AND WASER, N. M. 1979. Pollen dispersal and optimal outcrossing in *Delphinium nelsonii*. *Nature*, **277**, 294–296.
- RITLAND, C. AND RITLAND, K. 1989. Variation of sex allocation among eight taxa of the *Mimulus guttatus* species complex. *Am. J. Bot.*, **76**, 1731–1739.
- SAS INSTITUTE 1990. *SAS/STAT User's Guide*, version 6, 4th edn. SAS Institute, Cary, NC.
- SCHAAL, B. A. 1980. Measurement of gene flow in *Lupinus texensis*. *Nature*, **284**, 450–451.
- SHORE, J. S. 1993. Pollination genetics of the common milkweed, *Asclepias syriaca* L. *Heredity*, **70**, 101–108.
- STEEL, R. G. D. AND TORRIE, J. H. 1980. *Principles and Procedures of Statistics: A Biometrical Approach*, 2nd edn. McGraw-Hill, New York.
- SUTHERLAND, S. AND VICKERY, R. K. 1988. Trade-offs between sexual and asexual reproduction in the genus *Mimulus*. *Oecologia*, **76**, 330–335.
- THOMSON, J. D. AND PLOWRIGHT, R. C. 1980. Pollen carry-over, nectar rewards and pollinator behavior with special reference to *Diervilla lonicera*. *Oecologia*, **46**, 68–74.
- THOMSON, J. D. AND THOMSON, B. A. 1989. Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. *Evolution*, **43**, 657–661.
- TURNER, M. E., STEPHENS, J. C. AND ANDERSON, W. W. 1982. Homozygosity and patch structure in plant populations as a result of nearest-neighbor pollination. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 203–207.
- WRIGHT, S. 1931. Evolution in Mendelian populations. *Genetics*, **16**, 97–159.
- WRIGHT, S. 1938. Size of population and breeding structure in relation to evolution. *Science*, **87**, 430–431.
- WRIGHT, S. 1946. Isolation by distance under diverse systems of mating. *Genetics*, **31**, 39–59.