High outcrossing rates maintain male and hermaphrodite individuals in populations of the flowering plant *Datisca glomerata*

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MODELS for the maintenance of androdioecy (the presence of male and hermaphrodite individuals in a breeding population) in plants predict that males must have a fertility at least double the male fertility of hermaphrodites in order to be maintained by selection[1–3]. An even greater advantage is required in partially self-fertilizing populations[1–3] as the gain in fitness through increased pollen production is least when few ovules are available for outcrossing. Because such stringent theoretical requirements make the evolutionary stability of this breeding system highly unlikely, functional androdioecy is thought to be rare in plants, and indeed the only documented instance occurs in populations of *Datisca glomerata* (Datiscaceae)[4]. As such, these populations provide a unique opportunity to test predictions concerning the evolution of androdioecy in plants. Here we report high outcrossing rates (65–92%) in two androdioecious populations of *D. glomerata* using random amplified polymorphic DNA markers. These

outcrossing rates, when analysed with respect to existing evidence concerning pollen production and inbreeding depression in this species, are sufficiently high to satisfy theoretical requirements for the maintenance of androdioecy.

A previous study[5] of genetic variation in *D. glomerata* using isozyme electrophoresis revealed an inbreeding coefficient (F) of 0.617 ($P<0.001$). This value of F suggests high rates of self-fertilization, whereas models for the maintenance of males in androdioecious populations require low rates. But as F is a product not only of self-fertilization, but of any type of non-random mating and selection, an estimate of cross-fertilization among genets (genetically distinct individuals, each arising from a single zygote) was needed to test theoretical models for the maintenance of androdioecy.

Outcrossing estimates can be generated by examining allelic variation over many loci in progeny arrays from plants allowed to pollinate naturally (open-pollination) and using a maximum-likelihood model to exclude progeny resulting from self-fertilization[6–8]. Initial investigations employing isozyme electrophoresis produced insufficient numbers of polymorphic loci with which to exclude progeny. Therefore, we have examined random amplified polymorphic DNA (RAPD)[9] loci in open-pollinated progeny arrays of *D. glomerata* to obtain multilocus estimates of outcrossing.

The progeny of 12 hermaphrodite plants from an androdioecious population of *D. glomerata* 6 km south of Islip Saddle, Los Angeles County, California (Mt Islip[4]) were grown to a size sufficient for total DNA isolation[10] from fresh leaves or whole seedlings. Maternal DNAs were isolated from dried green capsular tissue. Families were surveyed for genetic polymorphism by DNA amplification and electrophoresis[11] of selected individuals. From a survey of 340 arbitrary 10-base oligodeoxynucleotide primers (primers 201–500, University of British Columbia Biotechnology Laboratory (UBC); primer sets C and D, Operon Technologies (OP)), we inferred 12 polymorphic loci (Table 1) which we used to determine multilocus single-family and population outcrossing rates (Table 2 and Fig. 1*a–c*). The multilocus outcrossing rate, t_m, can range from 0 to 1 and when multiplied by 100 represents the estimated percentage of genet-to-genet cross-fertilizations within a population. We estimated t_m with a maximum likelihood algorithm[12] modified to allow inclusion of dominant markers. All dominant loci were considered diallelic, thus all individuals lacking a fragment were assumed to be homozygous recessive for the same allele.

For the Mt Islip population, t_m was 0.92 (s.e.m. = 0.03). The predicted frequency of male plants in androdioecious populations under conditions of equal male and female zygote fitness is given by

$$q=\frac{t-2lv(t+i-ti)}{2iv(1-t)(1-l)+t(1+v-2lv)} \qquad (1)$$

where t is outcrossing rate, l is relative (female/male) pollen fecundity, i is relative (inbred/outbred) fitness of seeds due to

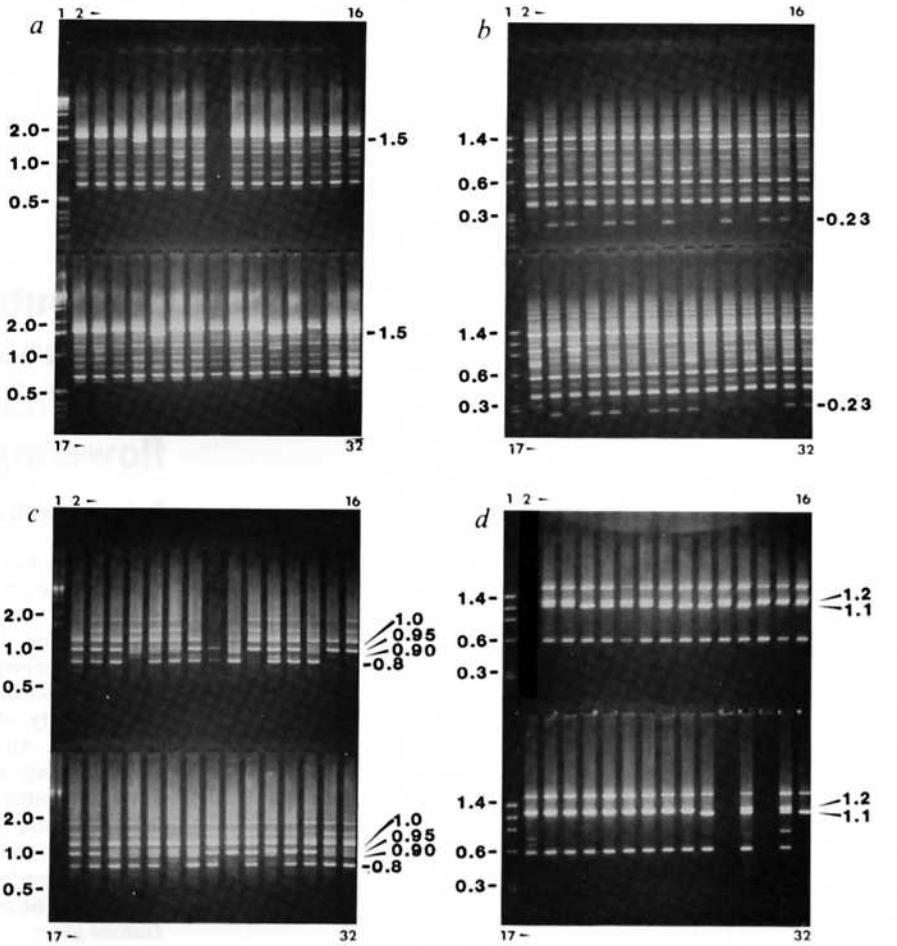

FIG. 1 RAPD profiles of selected families used in the estimation of outcrossing rates in *D. glomerata*. *a*, Mt Islip families 9, 10 and 11 amplified with Operon primer C-19. Lanes 1 and 17, kb ladder (Gibco BRL); lanes 2–7, family 9 (partial array; maternal parent not shown); lanes 8–16, 18–20, family 10 (maternal parent, lane 8); lanes 21–32, family 11 (maternal parent, lane 21). Progeny in lanes 5, 12, 22, 28, 29, 31 and 32 can be excluded from self-fertilization on the basis of the presence of the 1.5 kb allele. *b*, Mt Islip family 2 amplified with UBC primer 287. Lanes 1 and 17, PhiX174 DNA–*Hae*III digest marker (New England Biolabs); lane 2, maternal parent; lanes 3–16 and 18–32, progeny. Progeny in lanes 3, 4, 6, 7, 9, 12, 14, 15, 19, 21, 22, 24–26, 31and 32 can be excluded from self-fertilization on the basis of the presence of the 0.23 kb allele. *c*, Mt Islip family 6 amplified with UBC primer 437. Lanes 1 and 17, 1 kb ladder (Gibco BRL); lane 2, maternal parent; lanes 3–16 and 18–32, progeny. The fragments at 0.90 kb and 0.95 kb represent codominantly inherited alleles at a single locus. The maternal parent is homozygous for the slow (0.95 kb) allele, thus excluding from self-fertilization the heterozygous progeny in lanes 5–7, 10, 14, 20–24, 28, 31 and 32; the fragment at 1.0 kb, which amplifies only when the progeny are heterozygous, is assumed to be artifactual. The dominant allele of another locus at 0.8 kb is heterozygous in the maternal parent, thus, genotypes of progeny with the 0.8 kb allele cannot be determined. *d*, Alder Creek families 1, 2 and 3 amplified with UBC primer 106. Lanes 1 and 17, PhiX174 DNA–*Hae*III digest marker (New England Biolabs); lanes 2–13, family 1 (maternal parent not shown); lanes 14–16 and 18–26, family 2 (maternal parent, lane 14); lanes 27–32, family 3 (partial array; maternal parent, lane 27). The fragments at 1.1 kb and 1.2 kb represent codominantly inherited alleles at a single locus. The maternal parent of family 1 (not shown) is homozygous for the fast (1.1 kb) allele, thus excluding from self-fertilization the heterozygous progeny in lanes 3, 4, 6–9 and 12. The maternal parent of family 2 is homozygous for the slow (1.2 kb) allele, as are all progeny, and therefore none can be excluded. The maternal parent of family 3 is homozygous for the fast allele, and therefore the heterozygous progeny in lanes 29 and 31 can be excluded.

TABLE 1 Polymorphic loci used to determine t_m in populations of *D. glomerata*

Locality/Primer	Locus	Allelic mobility (kb)
Mt Islip		
OP C-19	1	1.5
	2	1.2
UBC 287	3	0.75
	4	0.23
UBC 300	1	1.8
UBC 341	1	1.8
UBC 376	1	0.80
UBC 437*	1	0.90, 0.95
	3	0.8
UBC 474	1	1.1
	2	0.72
UBC 490	1	0.9
Alder Creek		
OP A-8*	1	0.92, 0.95
UBC 104*	1	1.36, 1.40
	2	1.0
	3	0.75
UBC 106*	1	1.1, 1.2
UBC 152	1	1.9
	2	1.7
	3	0.6
UBC 174	1	1.05
	2	0.7
UBC 287	1	1.6
	2	1.0
	3	0.75
UBC 341	1	1.8
UBC 409*	1	0.58, 0.59
UBC 413	1	0.8
UBC 437	2	0.85
UBC 474	2	0.72
	3	0.70

For each primer, amplification products representing polymorphic loci are ordered arbitrarily by size; the largest fragment is designated as locus 1. Codominance was confirmed in all observed cases by excising amplification products representing each suspected codominant allele from the gel exhibiting the RAPD profile, cleaving each with a series of restriction endonucleases, and observing congruent band profiles OP, Operon Technologies; UBC, University of British Columbia; kb, kilobases.

* Codominant allelic expression.

TABLE 2 Single family and population outcrossing estimates in *D. glomerata*

Locality/Family	*N*	$t_m \pm$ s.e.m.
Mt Islip		
1	29	0.85 ± 0.08
2	29	0.86 ± 0.13
3	30	1.04 ± 0.20
4	28	1.10 ± 0.19
5	27	0.93 ± 0.31
6	31	1.01 ± 0.33
7	10	0.80 ± 0.33
8	9	1.00 ± 0.00
9	10	1.00 ± 0.00
10	11	0.78 ± 0.33
11	11	0.93 ± 0.54
12	11	0.56 ± 0.14
Population	236	0.92 ± 0.03
Alder Creek		
1	11	0.64 ± 0.25
2	11	0.28 ± 0.15
3	11	0.99 ± 0.55
4	11	1.00 ± 0.00
5	11	1.00 ± 0.00
6	11	0.84 ± 0.62
7	9	1.00 ± 0.00
Population	75	0.65 ± 0.05

N, number of progeny in each family; t_m, multilocus outcrossing rate estimate. Standard errors are each based on 100 bootstraps.

inbreeding depression, and v is relative (female/male) viability of zygotes to reproductive maturity[2]. Substituting our value of t_m for t, 0.26 for l (ref. 4), 0.86 for i (ref. 13; the estimate of i is based only on early phases of the life cycle, but in any case has little influence on q relative to t and l) and assuming $v = 1$ (no data), then $q = 0.28$. As $q > 0$, our estimate of t satisfies theoretical requirements for the maintenance of androdioecy in this population.

To confirm high outcrossing in androdioecious populations of *D. glomerata*, we estimated t_m for another androdioecious population 7 km south of Roundtop summit, Los Angeles County, California (Alder Creek[4]). From a survey of 163 primers (UBC 101–200, OP sets A and B, plus 23 robust primers from the Mt Islip survey), we inferred 19 polymorphic loci (Table 1) which were subsequently used to determine t_m (Table 2 and Fig. 1*d*). Only 3 of the 12 loci that were polymorphic in Mt Islip were detected as polymorphic in Alder Creek. In this population, t_m was 0.65 (s.e.m. = 0.05). Substituting t_m for t in equation (1) and keeping the other parameters the same, $q = 0.11$. Once again, outcrossing rates are sufficiently high to account for the maintenance of androdioecy.

The observed frequency of males in the two populations is $q = 0.24$ for Mt Islip and $q = 0.17$ for Alder Creek[4]. The Mt Islip value is 0.02 units lower than the q calculated using the lower limit of the standard error for t_m; the Alder Creek value is 0.02 units higher than the q calculated using the higher limit of the standard error for t_m. Nevertheless, observed and expected values of male frequency are surprisingly close, and indicate that at least some populations of *D. glomerata* have male frequencies in equilibrium with an androdioecious breeding system; apparently because hermaphrodite plants have only about one quarter of the male fertility of male plants.

We suggest that the differences in outcrossing rate observed between Mt Islip and Alder Creek are density dependent. Mt Islip, with higher values of q and t, is a compact population, with inflorescences from several individuals often adjacent or touching, whereas Alder Creek, with lower values of q and t, is a sparse, scattered population. This hypothesis will be tested by conducting detailed mapping studies in conjunction with paternity exclusion analysis for several androdioecious populations with density differences.

The extreme rarity of androdioecy in plants seems to be paralleled by the rarity of comparable breeding systems in animals. Mixed populations consisting of self-fertilizing hermaphrodites and males have been documented only in the shrimp genera *Eulimnadia*[14] and *Triops*[15], and probably occur in the barnacle genus *Iblaquadravalvis* (P. Raimondi, personal communication). Comparison of the natural history of *D. glomerata* to these vastly different organisms may provide additional insights regarding the evolutionary circumstances under which androdioecy originates and is maintained.

In addition to confirming theoretical models for the maintenance of androdioecy, this study demonstrates the utility of RAPD markers in outcrossing rate estimations, especially when isozymes fail to provide sufficient polymorphism. For future outcrossing studies using RAPD technology, we suggest the development of codominant loci using restriction endonuclease cleavage of products amplified with RAPD primers, because dominant markers, which constitute about 95% of all RAPD markers obtained[9,11], yield little information if present in the maternal parent. This will result in the need for fewer numbers of polymorphic loci for reliable multilocus outcrossing

estimates. Codominant markers are currently being developed for populations of *D. glomerata.* □

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