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Numbers of sporophytic self-incompatibility alleles in populations of wild radish

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Summary. To estimate the numbers of sporophytic *S*-alleles in two adjacent populations of wild radish, we performed 701 reciprocal crosses among 50 individuals. Each cross was replicated five times in each direction. Sixteen plants were fully intercompatible, indicating the presence of at least 32 *S*-alleles in the two populations. A minimum of 22 *S*-alleles occur in a single population. The frequency of incompatibility was significantly higher for within-population crosses (14.5%) than for between-population crosses (7.8%). This suggests that the two populations differ in the composition and frequency of alleles at the *S*-locus.

Key words: Sporophytic self-incompatibility – Plant breeding systems – *Raphanus sativus* – Multiple paternity

Introduction

Population genetic theory suggests that frequency-dependent selection and gene flow can maintain large numbers of self-incompatibility alleles in plant populations (Wright 1939, 1964; Bateman 1947; Imrie et al. 1972; de Nettancourt 1977; Richards 1986). Estimates of the minimum number of *S*-alleles in populations of angiosperms with gametophytic incompatibility support the theory. In several cases, tens to hundreds of *S*-alleles are present in populations (e.g., Emerson 1939; Atwood 1944; Williams 1947). By contrast, there are few data available on numbers of *S*-alleles in populations of species exhibiting sporophytic incompatibility. One of the only detailed studies is that of Bateman (1954), who estimated that at least 22 *S*-alleles were present in a large population of *Iberis amara* from Hertfordshire, UK. A second study, by Sampson (1967), identified *S*-alleles from three *Raphanus raphanistrum* populations in eastern Canada and two populations in Poland. Thirteen or fewer *S*-alleles were found in each population.

In this paper we describe an investigation of the number of *S*-alleles in two adjacent populations of wild radish (*Raphanus sativus* L). This cosmopolitan, annual species typically grows along roadsides and on agricultural land. Both *Raphanus sativus* and closely related *Raphanus raphanistrum* exhibit homomorphic sporophytic incompatibility, with multiple *S*-alleles present at a single locus (Bateman 1955; Sampson 1957, 1964, 1967). A recent study by Lewis et al. (1988) indicates that an ancestral gametophytic locus is also present. However, in most cases, patterns of incompatibility are adequately explained by the sporophytic locus.

In addition to estimating the minimum number of *S*-alleles in the two populations, we compare the proportion of compatible matings within and among populations. This allows us to determine whether the populations are genetically differentiated at the *S*-locus. The data are then interpreted in light of recent studies of gene flow and patterns of paternity in these and nearby populations.

Materials and methods

Seeds were randomly harvested from 50 maternal plants in each of two adjacent populations (designated A and B) in Riverside/CA. Two plants from each family were grown in a pollinator-free greenhouse at the University of New Mexico. The plants were raised in 15.6-cm diameter round plastic pots, which contained a mixture of 4 parts sand, 1 part peat, and 1 part perlite. They were watered daily and fertilized weekly with a dilute solution of Peter's 20:20:20 NPK and Peter's soluble trace element mix.

As the plants began to bloom, they were tested for self-compatibility, and self-compatible individuals were discarded. Self-

Table 1. Cross-compatibility of 50 *Raphanus sativus* plants from two populations. Compatible crosses are designated +, incompatible crosses are indicated with a black square. When a cross was not attempted, a blank space is present. Prefix to plant number indicates origin of seed: A – population A, B – population B, a – population A from an earlier year. Plants are listed in the order that crosses were performed

	A02	A04	A07	A10	A15	A31	A34	A36	A42	B11	B31	B33	B36	B40	a27	a46	a55	a35	A24	B05	B03	A09	B01	A16	A25	A30	A38	B13	B18	B25	B32	A20	A26	A27	B49	a29	A01	B48	A05	A06	A08	A23	A44	B21	A17	A29	A40	A43	A21	B23
A02		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A04	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	■	+	+	+	+	+	+	+	+	+	+	+	+	+	■	+	+	+	+	+	■	+	+	+	+	+	+	+	+
A07	+	+		■	+	+	+	■	+	+	+	+	+	+	+	+	+	■	+	■	+	+	■	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	■	+	+	+	+		+	+	+	+	+	+
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A34	+	+	+	+	+	+		+	+	+	+	■	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	■	+	+	+	+	+	+	+	■	+	+	+	+	+	■	+	+	+	+	+	+
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B11	+	+	+	+	+	+	+		+		+	+	+	+	■	+	+	+	+		+		+	+	+	+	■	■	+	+	+		+	+	+		+	+		+		+	■		+		+	+		+
B31	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	■	■	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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B40	+	+	+		+	+	+	+	+	+	+	+	+		+	+	+	+	■	+	■		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	■
a27	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+		+	+	+	+	+	■	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
a46	+	+	+			+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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B48	+	+	+			+	+		+	+	+		+	+	+	+	+				+			+		+			+		+		+	+	+		+		+	+	+	+	+	+	+	+	+	+	+	+
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A08	+	+	+			■	+		+	+	+		+	+	+	+	■				+			+		+			+								+	+	+	+		■	+	+		+	+	+	■	+
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A44	+	+	+			+	+		+	■	+		+	+	■	+	+				+			+		■			+		+		+	+	+		+	+	+	+	+	+		+	+		+	+	+	+
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compatibility is extremely rare in these two populations. We then haphazardly selected self-incompatible plants, using only one per family, for inclusion in the experimental crossing design. Initially, 15 plants were crossed in a complete diallel. Since dominance relationships often differ in the pollen and the style (Bateman 1954; Thompson and Taylor 1966; Ockendon 1974; de Nettancourt 1977; Wallace 1979), we performed reciprocal crosses to maximize the opportunity of detecting cross-incompatibility. Five replicates of each cross were performed by brushing a dehiscing anther across a receptive stigma. Pedicels of pollinated flowers were then marked with small paper tags.

Compatible crosses were determined by censusing fruit set, an approach similar to that of Sampson (1957). We used this method, rather than scoring stigmas for pollen tube penetration, because we needed to produce fruits for an unrelated experiment requiring a diallel crossing design. However, other workers have found that the two methods give similar results (Bateman 1954; Wallace 1979). Fruit production was censused 7 days following hand-pollination, and no fruit abortion was observed after this time. A cross was scored as compatible if four or five fruits were developing, and incompatible if zero or one fruit was present. In the few cases where two or three fruits were recorded, five additional replicate pollinations were performed. If less than four of the five additional pollinations resulted in fruits, the cross was scored as incompatible.

Of the 15 plants initially tested, 9 were fully intercompatible. Additional plants were then reciprocally crossed with these 9 individuals. When newly tested plants were compatible with these individuals, they were added to the core group. Because plants from population A tended to bloom earlier than plants from population B, more individuals from A were included in the crossing design. An additional five families, derived from site A in a previous season, were tested in a preliminary effort to address whether the composition and frequency of *S*-alleles changes over time. Data for these plants were included in overall analyses, but were excluded from comparisons within and among populations. A total of 50 plants was screened for self-incompatibility alleles, requiring more than 7,000 hand-pollinations.

Results

Of the 701 reciprocal crosses, 624 (89.0%) were compatible (Table 1). In the majority (58.0%) of cases of cross-incompatibility, both directions of a reciprocal cross were unsuccessful. Overall, fewer than 5% of all pairs of crosses yielded nonreciprocal results.

Of the 50 plants tested, 16 were fully intercompatible. Since sporophytic incompatibility is based on the diploid genotypes of seed and pollen parents, and nearly all individuals are heterozygous (de Nettancourt 1977), a minimum of 32 *S*-alleles are present in the two populations. In the more intensively sampled population (A), 11 of 29 plants were intercompatible, indicating the presence of at least 22 *S*-alleles.

The frequency of incompatibility was higher for within-population crosses (14.5%) than for between-population crosses (7.8%) (Table 2). This comparison was tested with a *G*-test of independence (Sokal and Rohlf 1981), and found to be significant ($G=6.0$, $df=1$, $p<0.02$).

Table 2. Frequency of cross-incompatibility within and between populations of *Raphanus sativus*. See text for statistical analyses

Type of cross	No. of reciprocal crosses	% incompatible crosses
Within population A (29 plants)	217	15.7
Within population B (16 plants)	73	11.0
Between populations (45 plants)	269	7.8
All possible combinations[a] (50 plants)	701	11.0

[a] This category includes 5 plants that were grown from seeds harvested in population A during a previous season

Discussion

The populations of *Raphanus sativus* in this study exhibit as many, or more, *S*-alleles as have been identified in other taxa with sporophytic self-incompatibility (Table 3). This is especially notable since we only sampled two populations, comprising a small fraction of the range of the species. However, our methods probably underestimate the number of *S*-alleles present, since some alleles may only occur in individuals that were not tested or in plants having a second allele that had already been sampled.

Nonetheless, our estimate of 32 *S*-alleles closely corresponds with theoretical predictions given the measured frequency of cross-compatibility. In an idealized population with no self-compatibility, no dominance, and equal allele frequencies, the observed 89% cross-compatibility would occur when approximately 30–40 *S*-alleles are present (Ockendon 1974).

The higher frequency of cross-incompatibility within populations (14.5%) than between populations (7.8%) suggests that the two populations differ in the composition and frequency of *S*-alleles. A similar pattern was observed in a study of a subdivided *Iberis amara* population. Bateman (1954) recorded a higher frequency of cross-incompatibility within subdivisions (11%) than between subdivisions (4.8%).

Although the two *Raphanus sativus* populations may be somewhat genetically differentiated at the *S*-locus, frequent exchange of *S*-alleles among populations is likely. Paternity exclusion analyses employing isozyme markers demonstrate that these and neighboring populations exhibit 5%–18% gene flow (Ellstrand and Marshall 1985; Ellstrand et al. 1989). This level of gene flow may help maintain large numbers of *S*-alleles within populations (Imrie et al. 1972).

Table 3. A summary of the minimum number of *S*-alleles reported for taxa with sporophytic incompatibility

Species	Source of material	Minimum no. of *S*-alleles	Reference
Brassica oleracea var. *acephala*	N cultivars not reported	28	Thompson and Taylor (1966)
Brassica oleracea var. *gemmifera*	16 cultivars	19	Ockendon (1974)
Brassica oleracea var. *italica*	7 cultivars	20	Ockendon (1980)
Iberis amara	1 population	22	Bateman (1954)
Raphanus raphanistrum	5 widely separated populations	13	Sampson (1967)
Raphanus sativus	2 adjacent populations	32	Present study

An interesting feature of *Raphanus* mating patterns may facilitate founding of populations with several *S*-alleles. Sampson (1967) demonstrated that the number of *S*-alleles from progeny of individual *Raphanus raphanistrum* fruits may exceed four, indicating the presence of two or more sires. Recent studies of *Raphanus sativus* indicate that multiple paternity within fruits frequently occurs. Seventy-five percent of 388 multiseeded fruits were sired by two to four pollen donors (Ellstrand 1984; Ellstrand and Marshall 1986). If an eight-seeded fruit were sired by four fathers, as many as ten *S*-alleles might be present in the progeny.

Because *Raphanus* fruits are indehiscent, siblings are likely to establish in close proximity. If such neighboring individuals shared the same sire, they would have a total of four *S*-alleles, and only 25% of within-family crosses would be compatible. By contrast, if a half-sib family had ten *S*-alleles, as many as 44% of within-family crosses would be compatible. Thus, when single fruits of this weedy species disperse to new habitats, multiple paternity may partially counteract the effects of sporophytic incompatibility, facilitating mating among siblings.

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