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# The breeding systems of six species of *Arabis* (Brassicaceae)[1]

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The ability of organisms to produce genetic variation for any trait, including resistance to pathogens, is partially determined by breeding system. I used enzyme electrophoresis, crossing experiments, and cytology to assess the breeding systems of co-occurring *Arabis* species that are often infected by rust fungi. The *Arabis holboellii* surveyed were pseudogamous apomicts with relatively high population-level allozyme variation, and variable chromosome numbers ($2n$, $3n$, $4n$). *Arabis gunnisoniana* ($3n$) and *Arabis lignifera* ($2n$) were also pseudogamous but showed no allozyme variation either at the population level, or within progeny arrays. *Arabis hirsuta* may be an autogamous polyploid or it may be a pseudogamous apomict; more work is needed to clarify the breeding system of this species. *Arabis drummondii* and *Arabis crandallii* were sexual, but exhibited little genetic variability due to a predominance of self-fertilization. The use of several techniques was necessary to evaluate these breeding systems. Insect exclusion indicated when pollen was necessary for seed set, but could not differentiate between sexual reproduction and pseudogamy. Electrophoresis yielded information on the degree of selfing (as evidenced by homozygosity) and apomixis (fixed heterozygosity), but could not differentiate between autogamy and apomixis in polyploids without allozyme variation. Pseudogamy was confirmed when crosses between dissimilar genotypes yielded only the maternal genotype, and cytologically by irregular meiosis.

The ability of any organism to produce genetic variation for any trait, including resistance to pathogens, is partially determined by breeding system; self-fertilization and apomixis yield little or no variation in progeny, while outcrossing among divergent genomes creates greater genetic variability (Bell, 1982). The *Arabis holboellii* complex, a group of ≈ 27 taxa (Rollins, 1941; Böcher, 1969), was considered by Böcher to be amphi-apomictic, because cytological and embryological studies indicated that some species were sexual, some were apomictic, and some such as *A. holboellii,* could have either sexual or apomictic reproduction, or even both on the same plant (Böcher, 1947, 1951, 1954, 1969). *Arabis* plants are often infected by systemic rust diseases (*Puccinia* spp.), which can cause severe reduction in survival and reproduction (Roy, 1993a; Roy and Bierzychudek, 1993). In western Colorado, it is common to find several species of *Arabis* co-occurring at the same locality, some infected and some not. As part of a broader study on the ecological and evolutionary consequences of rust disease in *Arabis* (Brassicaceae), I have undertaken a study of the breeding systems of six *Arabis* species. Five species are members of the *Arabis holboellii* complex (= series 2 of Rollins [1941]): *A. crandallii* Robinson, *A. drummondii* Gray, *A. gunnisoniana* Rollins, *A. holboellii* Hornem, and *A. lignifera* Nelson. The sixth species, *A. hirsuta* (L.) Scopoli, is a member of the *A. hirsuta* complex.

I used a combination of enzyme electrophoresis, crossing studies, and cytology to determine breeding systems. Electrophoresis was used to check progeny arrays for segregation of alleles and to measure the degree of heterozygosity within populations (Ritland, 1983). Selfing taxa are typically homozygous at many loci, but there are occasional heterozygotes and these should segregate in progeny arrays (Gottlieb, 1981). In selfing plants there tends to be more genetic variation among populations than within populations because different alleles are fixed during inbreeding (Crawford, 1983; Barrett and Shore, 1989). Obligately apomictic taxa are typically heterozygous at many loci (Richards, 1986), there is no segregation of alleles in progeny arrays, and all progeny from the same mother should be genetically identical (Bierzychudek, 1985; Ellstrand and Roose, 1987). As in self-fertilizing species, the majority of genetic variation in apomictic taxa is typically among, not within populations (Levin and Kerster, 1971). Finally, outcrossing taxa can be diagnosed using allozyme data. Outcrossers should have both heterozygous and homozygous loci, and there should be segregation of alleles in the progeny. Outcrossing species usually have as much or more allelic variation within populations as among populations (Gottlieb, 1981).

Identifying apomixis by means of enzyme electrophoresis is not always as simple as checking for fixed heterozygosity. Some apomicts such as *Eupatorium* are not highly heterozygous (Yahara, 1990), thus it is important to verify breeding systems based on allozyme analysis by doing cytology and by performing crosses. Another complication is that it is possible for selfing polyploids to yield a pattern of fixed heterozygosity that is similar to that shown by apomicts (e.g., *Turnera*; Barrett and Shore, 1989). When fixed heterozygosity is encountered in a polyploid species further experimentation is necessary to differentiate autogamy from apomixis. Unfortunately, testing whether or not pollen is required for seed set is insufficient to distinguish selfing polyploids from apomicts (which are also often polyploid) because in one common form of apomixis called pseudogamy, pollen does not fertilize the egg, but pollen is required to fertilize the

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endosperm (Grant, 1981; Richards, 1986; Mogie, 1992). To differentiate pseudogamy from autogamy in polyploids, I devised experimental crosses in which the pollen donor was of a different multilocus genotype than that of the female recipient. The progeny of such crosses were examined by electrophoresis. If there was no segregation, and the progeny showed only the female's allozyme genotype, then pseudogamy was indicated and not autogamy.

## MATERIALS AND METHODS

***Localities***—All populations were located within Gunnison County, Colorado. The Cement Creek site is a rocky meadow to the north of the Cement Creek Road (forest route 740), 4.0 km from its intersection with highway 135. The Gold Creek site is a meadow 1.0 km north of Ohio City to the East of the Gold Creek road. The Taylor River and Almont Triangle sites are, respectively, 7.2 and 8.1 km up the Taylor River road from its intersection with highway 135. The Gothic site is a large meadow 1.0 km north of the Rocky Mountain Biological Laboratory near the East River.

***Cytology***—Chromosome counts were made for *A. holboellii, A. lignifera,* and *A. gunnisoniana.* Flower buds were collected from greenhouse grown plants into modified Carnoy's solution (Carr and Kyhos, 1981) between 0900 and 1100 in the morning, the buds were then left at room temperature for 4 d, then transferred to a freezer until used. Anthers were dissected in aceto-carmine for chromosome staining and permanently mounted in Hoyer's solution (Beeks, 1955). Counts were made from at least five cells per individual.

Several other characteristics associated with cytology and fertility were measured. The configuration of the pollen (monads, dyads, or tetrads) was determined. I checked pollen viability by staining pollen cells with cotton blue (Maneval, 1936), then calculated the percent of stained cells in a sample of 300. To test for seed sterility, I germinated 66–418 seeds from each parent on soil (1 part peat: 1 part sand: 1 part vermiculite) in individual wells of seedling trays. Finally, to determine whether pollen size was correlated with ploidy level, I measured the diameter of 25 randomly selected pollen grains for each individual from which a chromosome count was obtained.

***Electrophoresis***—Tissue for electrophoresis came from a combination of field-collected leaf material and from leaves of plants propagated in the greenhouse. In either case, the initial collection procedures were the same: a transect was laid through a natural population and seeds or leaf tissue were collected from the plant nearest to the line at each even meter mark. Field-collected leaves were either lyophilized immediately or were kept on ice for 2 d during transportation from the field sites to the laboratory, then transferred to an ultralow freezer (−80 C) until use. Seeds were sown into individual pots in a greenhouse in a soil mix of sand, pea gravel, peat, and vermiculite (1:1:1:1 ratio). All three treatments of leaves (fresh, lyophilized, and fresh-frozen in ultralow freezer) yielded good results, but the best results were from fresh-frozen material stored in the ultralow freezer.

Leaf tissue was homogenized in the tris-HCl grinding buffer of Soltis et al. (1983) using 3 g of polyvinylpyrrolidone (PVP), then the homogenate was absorbed onto paper wicks and run on 12% starch gels. Six enzymes were resolved using gel and electrode buffer system 8- of Rieseberg and Soltis (1987): acid phosphatase (AcPh, 1 locus), aspartate aminotransferase (AAT, 3 loci), fluorescent esterase (FE, 3 loci), leucine aminopeptidase (LAP, 2 loci), phosphoglucoisomerase (PGI, 2 loci), and triosephosphate isomerase (TPI, 2 loci). System 5 of Soltis et al. (Soltis et al., 1983) was used for aconitase (ACN, 2 loci), isocitrate dehydrogenase (IDH, 2 loci), malic enzyme (ME, 1 locus), 6-phosphogluconate dehydrogenase (6PGD, only locus 2 was scored because locus 1 was often poorly resolved), and shikimic dehydrogenase (SkDh, 1 locus). All enzyme stains followed Soltis et al. (1983) except LAP, which was modified by the addition of 30 mg of L-leucine-beta-naphthylamide.

Loci were assigned on the basis of segregation in the two sexual species, *A. drummondii* and *A. crandallii,* and by comparison with typical diploid patterns (in plants) for numbers of isozymes, subcellular compartmentalization and quaternary structure of the enzymes (after Gottlieb, 1982; Wendel and Weeden, 1989). In all cases, the fastest migrating isozyme was designated as 1, with slower isozymes given sequentially higher numbers. Alleles were also sequentially named, starting with the letter a. For multimeric enzymes, bands detected in the polyploid taxa that were not also found in the diploid sexual species, were designated as intra- or intergenic heteromers unless they were at the top or bottom of the gel, or represented the only band observed in an individual at a specific locus, in which case they were designated as allozymes.

Allele frequencies were only calculated for taxa that segregated in typical diploid sexual fashion. It was too difficult to score the other taxa for allele frequencies because of gene duplications that produced overlapping bands and unbalanced (asymmetric) heterozygosity (bands varying in staining intensity due to differences in gene dosage). In sexual species, crossing experiments make it possible to assign alleles and loci because there is segregation in the progeny. Crosses cannot be made in apomicts. If the chromosome number is known, numbers of alleles can be estimated for an apomict by assuming a particular mode of inheritance (Bayer, Ritland, and Purdy, 1990). However, chromosome numbers in *Arabis* populations are highly variable (Rollins, 1941; Böcher, 1951, 1954, 1969), thus each individual surveyed for allozymes would also need to be checked cytologically, an impossibility for population surveys. It is possible, however, to determine presence or absence of bands by running apomicts side by side in gels with sexual species, as was done during this study.

Heterozygosity was assessed three different ways: for the diploid sexual taxa I calculated $H_o$ (observed heterozygosity) and $H_e$ (expected heterozygosity), and for the putative apomicts I calculated the percentage of loci that exhibited fixed heterozygosity (heterozygous loci remain heterozygous in progeny arrays). Percent polymorphic loci ($P$) and the mean number of alleles per locus ($A$) were calculated from all loci (both polymorphic and monomorphic) for all populations and taxa. A locus was considered polymorphic if the most common allele had a

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TABLE 1. Summary of cytological observations.

| Species | Genotype[a] | Site of origin | % germ of seeds (*N*) | Chromo. # | Pollen config. | Stainable pollen (%) | Pollen diam (μm) |
|---|---|---|---|---|---|---|---|
| *A. holboellii* | A | Cement | 46.0 (66) | 28 | Dyads | 85.0 | 26.6 ± 2.35 |
| | A2 | Taylor | 56.0 (66) | 21 + f | Dyads | 88.0 | 27.7 ± 2.29 |
| | A3 | Taylor | 57.0 (66) | 21 | Dyads | 86.0 | 32.6 ± 2.22 |
| | A15 | Taylor | 83.3 (66) | 21 + f | Dyads | 95.0 | 20.7 ± 2.7 |
| | A22 | Taylor | 46.0 (66) | 21 + f | Dyads | 88.0 | 30.3 ± 2.06 |
| | A24 | Taylor | 15.0 (66) | 21 | Dyads | 3.0 | 20.5 ± 1.76 |
| | B1 | Taylor | 46.0 (66) | ? | Monads + dyads | 60.0 | 29.4 ± 1.89 |
| | B11 | Gold | 32.0 (66) | 14 | Dyads | 86.0 | 28.2 ± 1.67 |
| | C5 | OneMile | 38.0 (352) | 21 + f | Dyads | 42.0 | 31.3 ± 4.35 |
| | D1 | Cement | 51.0 (418) | 14 | Dyads + tetrads | 92.0 | 28.2 ± 2.73 |
| | E6 | Cement | 71.0 (66) | 21 | Dyads | 97.0 | 28.0 ± 2.45 |
| | F2 | Cement | 77.0 (352) | 21 | Dyads | 73.0 | 31.6 ± 4.62 |
| *A. gunnisoniana* | A9 | Taylor | 38.0 (66) | 21 | Dyads | 58.0 | 30.8 ± 2.4 |
| *A. lignifera* | C | Taylor | 46.0 (66) | 14 | Tetrads | 91.0 | 26.0 ± 2.4 |

[a] Descriptions of the allozyme genotypes and data on their abundance at the different sites can be found in Roy (1993b). Many of these genotypes are present at more than one site. The site of origin column reflects the site from which cytological material was collected and not necessarily the known range of the genotype.

frequency of < 0.95. The estimates of *A* and *P* are probably underestimates for the polyploid species because each band was weighted evenly and given a score of one, despite the possibility that more than one allele may have contributed to it.

Breeding systems were determined, in part, by assessing population variation within a random sample, and by testing natural progeny arrays. One to four families with 8–28 members each were tested for each species, except for *Arabis holboellii,* for which the results of 20 progeny arrays were previously documented (Roy and Rieseberg, 1989). A subset of enzymes, some consistently polymorphic ones (PGI, TPI, LAP, IDH, and ME), was used for screening the families. Somewhat different subsets of enzymes were scored for the progeny arrays,but a minimum of five polymorphic loci were used per analysis.

***Crossing experiments***—Individuals of *Arabis holboellii, A. lignifera,* and *A. gunnisoniana* revealed fixed heterozygosity at many allozyme loci. To test for pseudogamy I made crosses among 23 plants of *A. holboellii* representing ten different multilocus allozyme genotypes, three plants of *A. gunnisoniana* representing one genotype (only one was found), and two plants of *A. lignifera* of one genotype (only one was found). The plants were grown in small individual pots (5.5 × 8.5 cm) randomly arranged on a single bench in the greenhouse in a soil mix of sand, pea gravel, peat, and vermiculite (1:1:1:1 ratio). When the plants were in bud I covered each inflorescence with a fine mesh bag that excluded insects but not light. I then randomly assigned 2–3 buds on each inflorescence to each of four different pollination treatments as follows:

1) Emasculation. Anthers were removed from closed buds by gently opening the buds with fine forceps and plucking the anthers out. Emasculation is the best way to test for autonomous apomixis (Richards, 1986). If seeds are produced without pollen, then pollen is not necessary and the seeds are apomictic. If no seeds are set, then either pollen is necessary or the flower was so traumatized by the treatment that it aborted. These possibilities can be ruled out by comparison of seed set in this treatment with that of the emasculated and outcrossed (#4) and selfed (#2 and #3) treatments. In all treatments requiring emasculation, I emasculated flowers 1 d before pollinating them because the anthers matured a little earlier than the stigmas did.

2) Emasculated and selfed. Flowers were emasculated, then selfed by removing anthers from another flower on the same plant, and applying these to the chosen stigma. This selfing treatment, in combination with the emasculated and outcrossed treatment (#4), tests for self-compatibility and pseudogamy. If seeds set, then the plants are either self-compatible or pseudogamous apomicts (pseudogamy from self-pollen has been documented [Nybom, 1989; Campbell and Dickinson, 1990]). Lack of seed set is more difficult to interpret. It could mean that my pollination technique was ineffective, or that I damaged the flowers, or that the plants were self-incompatible. To differentiate among these possibilities it was necessary to compare seed set in this treatment with the naturally selfed treatment (#3) and with the emasculated and outcrossed treatment (#4).

3) Naturally selfed (= within plant controls). These were unmanipulated flowers and their seed set was compared with all other treatments. If, for example, seed set was less in the treatment where I manually self-pollinated flowers (#2), than it was here, then there was evidence that I damaged the flowers during manual pollination.

4) Emasculated and outcrossed. Flowers were emasculated, then pollen from a donor of a known different allozyme genotype was applied to the stigma. Two kinds of crosses were made, those using interspecific pollen and those using intraspecific pollen. I used intraspecific pollen when there was allozyme variation within a species, such as in *A. holboellii.* I used interspecific pollen when there was no detectable allozyme variation among individuals of a species, such as in *A. lignifera* and *A. gunnisoniana.* The emasculated and outcrossed treatment, in combination with the emasculation treatment (#1) and the selfed treatments (#2, and #3), tested for evidence of self-incompatibility and for pseudogamy.

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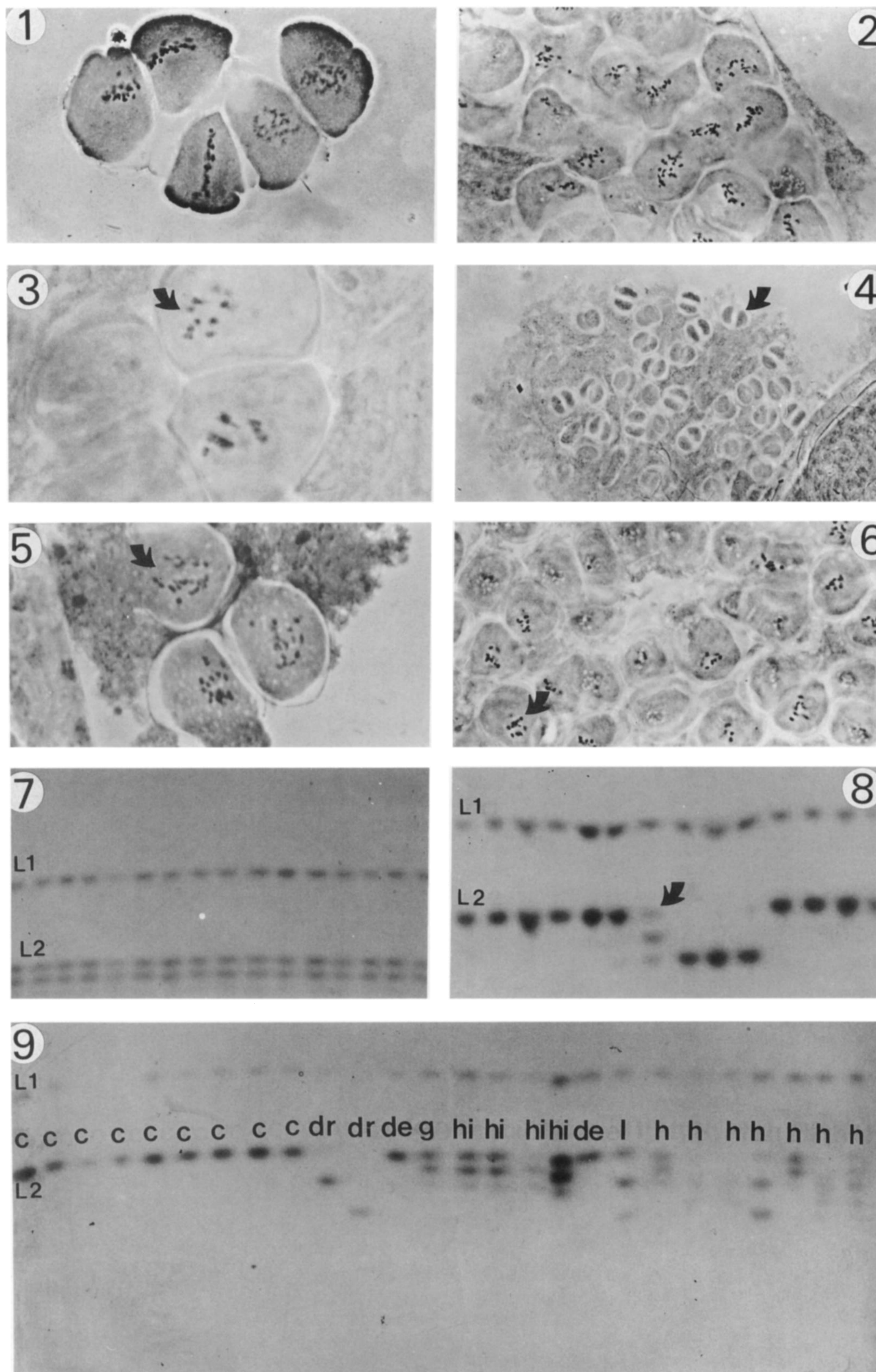


Figs. 1–9. Chromosome numbers and allozyme variation in *Arabis*. Note that all of the chromosomes present in a cell may not show up in the photographs due to unusually "sticky" chromosomes that do not separate well (D. Kyhos, personal communication), and loss of depth of field at high magnification. **1.** *Arabis holboellii,* triploid ($2n = 21$) cells of genotype A (see I, lanes 20 and 24 for the genotype "A"). **2.** *Arabis holboellii,* triploid ($2n = 21$) cells of genotype F2 mostly in metaphase (see Fig. 9, lane 26 for the genotype F2). **3.** *Arabis holboellii,* diploid ($2n = 14$), cells

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## RESULTS

***Cytology***—There is considerable variation in chromosome number within *Arabis holboellii* (Table 1). It is even possible to find diploid, triploid, and tetraploid individuals within the same population (Cement Creek, Table 1). The most common chromosome number for *A. holboellii* is the triploid 21 (8/12 individuals in Table 1), and the most common pollen configuration was dyads (12/12 individuals in Table 1; Fig. 4). Normal pollen tetrads were only associated with diploid plants, but some individuals with diploid numbers also had abnormal pollen formation (dyads) and irregular meiosis (Table 1; Fig. 4). Pollen sizes were variable and not consistent with ploidy level, for example, the diameter of triploid pollen spanned the whole range of pollen sizes (Table 1). The majority of individuals tested were highly fertile (Table 1). The one exception, genotype A24, was very close to sterile with seed germination at 15% and pollen viability of 3% (Table 1).

*Arabis gunnisoniana* was triploid (Table 1; Fig. 5). By comparison with *A. holboellii, A. gunnisoniana* showed little pollen stainability and poor seed germination (Table 1; Fig. 5). The one individual counted of *A. lignifera* was diploid, $2n = 14$ (Table 1; Fig. 6).

***Electrophoresis***—A list of populations surveyed, the percent polymorphic loci per population, and the mean number of alleles per locus, and measures of heterozygosity, are presented in Table 2. A summary of the allele frequencies and banding patterns from the allozyme survey of populations from six species of *Arabis* is given in Table 3. Nineteen to 20 putative loci were observed, with only five of these being monomorphic across all taxa (AAT 2, 6PGD 2, FE 3, LAP 2, and PGI 1). Figure 9 shows a representative sample of PGI banding patterns for each species surveyed. Note that PGI 1 (labeled L1) is monomorphic across all species, whereas PGI 2 (labeled L2) is polymorphic. Allelic frequencies are reported for the putative sexual species, *A. drummondii* and *A. crandallii,* in Table 3, and the percent of individuals surveyed showing a particular band are reported for the putative apomictic taxa (*A. gunnisoniana, A. hirsuta, A. holboellii,* and *A. lignifera*). The reason for this difference in reporting is that in the polyploid apomictic taxa it is impossible to determine the number of alleles contributing to bands because crosses cannot be made. It is possible, however, to identify shared bands and the probable position of loci by running all of the species side by side on gels. I drew on this information to create Table 3.

The taxa can be grouped by the degree of allelic variation, as indicated by mean number of alleles per locus and the percentage of polymorphic loci (Table 2), and by whether or not the progeny arrays showed fixed heterozygosity (Table 2). Populations of *Arabis drummondii* (Fig. 8) and *A. crandallii* (Fig. 9, lanes 1–9) had few alleles, the fewest polymorphic loci, and no fixed heterozygosity. *A. gunnisoniana* (Fig. 7) and *A. lignifera* (Fig. 9, lane 19) also had few alleles, but were more heterozygous than *A. drummondii* and *A. crandallii,* and progeny arrays of *A. gunnisoniana* and *A. lignifera* showed fixed heterozygosity at several loci. *A. holboellii* (Fig. 9, lanes 20–26) and *A. hirsuta* (Fig. 9, lanes 14–17) had the greatest number of alleles per locus, were the most polymorphic, and showed the highest levels of fixed heterozygosity.

***Crossing experiments***—Earlier studies (Roy and Rieseberg, 1989; Roy, 1993b) indicated that the Cement Creek population of *Arabis holboellii* was apomictic. Nonetheless, because pollen viability was high (Table 1) and because pseudogamous apomixis has been reported from this species in Greenland and Canada (Böcher, 1951), I also did a crossing experiment to test for pseudogamy. As is expected in pseudogamous apomicts, pollen was usually necessary for seed set in *A. holboellii.* Flowers set the most seed when they were bagged but otherwise unmanipulated (selfed mean $\pm$ SE = $15.0 \pm 2.8$, $N = 47$), the next most when emasculated and selfed ($9.0 \pm 2.0$, $N = 47$), a few when emasculated and pollinated with outcross pollen ($5.0 \pm 1.3$, $N = 47$), and the least when no pollen was applied (emasculated mean $\pm$ SE = $0.2 \pm 0.2$, $N = 47$). To differentiate polyploid selfing from pseudogamy, I grew seedlings from the successful outcross treatments involving electrophoretically different parents and tested these progeny for segregation of parental alleles. In all, 13 progeny arrays with a mean of nine sibs/family were tested. All progeny from each mother bore the same electrophoretic phenotype as their mother, as is expected in apomixis. Since pollen was necessary for proper seed set, the kind of apomixis is pseudogamy.

Electrophoresis of populations and progeny arrays indicated that *A. lignifera* may also be apomictic or pseudogamous since it has multilocus fixed heterozygosity. The crossing experiments indicate pseudogamy since pollen is required for fruit set (mean $\pm$ SE for four flowers/treatment: emasculated = 0, selfed = $11.06 \pm 6.0$, selfed + emasculated = $14.0 \pm 4.5$, outcrossed with *A. holboellii* pollen = $0.3 \pm 0.3$), and progeny tests showed that all the progeny resulting from crosses between unlike parents were identical to their mothers. Because I have been unable to detect electrophoretic variation in *A. lignifera,* I could not make crosses between known different electro-

←

from a genotype D1 plant in anaphase I at arrow (see Fig. 9, lanes 21, 22, 25 for the corresponding genotype). **4.** *Arabis holboellii,* dyads from a diploid individual of genotype D1, example dyad at arrow. **5.** *Arabis gunnisoniana,* triploid cells ($3n = 21$), at arrow note three groups of chromosomes. **6.** *Arabis lignifera,* diploid cells ($2n = 14$), metaphase II at arrow. **7.** Enzyme electrophoresis gel stained for PGI of a population sample of *Arabis gunnisoniana* showing no population variation. **8.** Enzyme electrophoresis gel stained for PGI of a population of *Arabis drummondii* showing two different homozygous bands at locus two (L2), and at the arrow, a heterozygote. **9.** Enzyme electrophoresis gel stained for PGI showing banding patterns for seven *Arabis* species. All species share the same monomorphic pattern at locus one (L1), but locus two (L2) is variable both within and among species. Lanes 1–9: c = *Arabis crandallii* Robinson, lanes 10–11: dr = *Arabis drummondii* Gray, lane 12: de = *Arabis demissa* Greene, lane 13: g = *Arabis gunnisoniana* Rollins, lanes 14–17: hi = *Arabis hirsuta* (L.) Scop., lane 18: de = *Arabis demissa* Greene, lane 19: l = *Arabis lignifera* A. Nelson, lanes 20–26: h = *Arabis holboellii* (Hornem.). The different genotypes for *A. holboellii* in lanes 20–26 show the PGI pattern observed in several commonly found genotypes (clones) described in Roy (1993b). Lanes 20 and 24 represent A, lanes 21, 22, 25 are from D1, lane 26 is from type F2.

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TABLE 2. List of species examined, voucher specimens, and summary of genetic variation within populations of six species of *Arabis* from Gunnison County, Colorado.

| Species | Population/Voucher[a] | $A$[b] | $P$[b] | $H_o$[b] | $H_e$[b] | $H$ (Fixed) |
|---|---|---|---|---|---|---|
| *A. crandallii* Robinson | Almont Triangle/Roy 1209 | 1.16 | 0.05 | 0.017 | 0.019 | 0 |
| *A. drummondii* Gray | Gold Crk./Roy 1199 | 1.16 | 0.11 | 0.006 | 0.043 | 0 |
| *A. drummondii* Gray | Gothic/Roy 1210 | 1.21 | 0.16 | 0.032 | 0.069 | 0 |
| *A. gunnisoniana* Rollins | Gold Crk./Roy 1201 | 1.21 | 0.21 | — | — | 0.21 |
| *A. hirsuta* (L.) Scop. | Gold Crk. | 1.60 | 0.45 | — | — | 0.45 |
| *A. holboellii* var. *retrofracta* (Grah.) Rydberg | Cement Crk./Roy 1191 | 1.55 | 0.45 | — | — | 0.45 |
| *A. lignifera* A Nelson | Cement Crk./Roy 1183 | 1.53 | 0.42 | — | — | 0.42 |

[a] Deposited at Rancho Santa Ana Botanic Garden, Claremont, CA.
[b] $A$ = the mean number of alleles per locus, $P$ = percent polymorphic loci, $H_o$ = observed heterozygosity for the sexual plants, and fixed $H$ = percent of loci that were fixed heterozygotes. $H_o$ and $H_e$ were not calculated for the putative apomicts because duplication and unbalanced heterozygotes made calculating allele frequencies problematic.

phoretic phenotypes to test for segregation in the progeny. Instead, I used pollen from a different species (*A. holboellii*) for the outcross treatments. One of the four crosses using *A. holboellii* pollen produced fruit, however, I could not determine whether pollen initiates seed production or whether it fertilizes the egg because only one seed was produced and the seedling generated from this seed did not survive long enough to genotype it with electrophoresis. The lack of seed set in the crosses suggests that *A. holboellii* pollen may not be a good substitute for *A. lignifera* pollen. Flowers of *A. lignifera* that were emasculated and then selfed produced abundant seed, suggesting that emasculation itself was not the reason why fruit set did not occur in the emasculation treatment.

Crossing experiments strongly suggest that *Arabis gunnisoniana* is pseudogamous. Flowers only set seed when pollen was present (mean ± SE for six flowers/treatment: emasculated = 0, selfed = 7.0 ± 5.3, selfed + emasculated = 13 ± 5.4, outcross = 13.0 ± 5.3) and progeny tests showed that all the progeny resulting from crosses between unlike parents were identical to their mothers. As in the experiments with *A. lignifera,* I used pollen from *A. holboellii* for the crosses to test for pseudogamy because there is no detectable isozyme variation in the populations of *A. gunnisoniana* that I have surveyed. Interestingly, in the case of *A. gunnisoniana,* intraspecific pollen from *A. holboellii* was perfectly capable of initiating seed set since 5/6 crosses produced seed. Moreover, this was a good test for pseudogamy since pollen was required to initiate seed set, but no fertilization took place as only the maternal allozyme genotype appeared in the 45 progeny tested.

## DISCUSSION

The six species of *Arabis* examined in this study can be described by different combinations of population variability resulting from their breeding system. *A. drummondii* showed the classical allozyme pattern for species that normally self-fertilize: individuals tended to be homozygous at every locus tested, and in a population survey very few heterozygotes were observed (see Fig. 8 at arrow for an exception). The observed number of heterozygotes was much smaller than expected for populations at Hardy-Weinberg equilibrium (Table 2), indicating inbreeding. The level of inbreeding was particularly high in the Gold Creek population (inbreeding coefficient $F = 0.86$ vs. 0.54 for the Gothic population). The low proportion of heterozygous loci found in *A. drummondii* (0.6–3%) is comparable to the 2.17% found in another selfing mustard, *Capsella bursa-pastoris* (reported by Richards [1986] from data compiled by Bosbach and Hurka [1981]). In addition to the population surveys, I examined four progeny arrays (mean of 12 sibs/family) generated from the seed of field-collected mothers of *A. drummondii*; the progeny were all homozygous at the same alleles as their mothers. To verify that the pattern of homozygosity indicated selfing, I prevented self-fertilization by emasculating flowers and then made crosses between two plants of *A. drummondii* known to have different alleles at the PGI-2 locus. As expected in the $F_1$, this cross yielded all heterozygotes. Sexuality in *A. drummondii* is also indicated by a lack of cytological abnormalities, and by diploid chromosome numbers (Rollins, 1941). Although *A. drummondii* can outcross, as evidenced by the occasional heterozygote in population surveys, and by my ability to create heterozygotes in crosses between unlike allozyme phenotypes, in nature it rarely outcrosses because self pollen is typically deposited on the stigma as the bud is opening.

Like *A. drummondii, A. crandallii* is most likely autogamous since there were few heterozygotes in the population surveys, and none in natural progeny arrays. Genetic diversity was low; by the standard cut-off for polymorphic loci, a frequency of 95% for the most common allele, only 5% of the loci in this population are polymorphic. Because there are few polymorphic loci, the number of expected heterozygotes is few, and it nearly matches the number observed (Table 2), leading to a low inbreeding coefficient (0.11), and suggesting little inbreeding. However, these statistics may be masking the possibility that in this population self-fertilization over time has led to the fixation of single alleles at most loci. This possibility is suggested by my observation in the field that these flowers normally self-pollinate as the bud opens, and by the high degree of selfing in another *Arabis* species, *A. drummondii* (see above). If fixation of alleles has taken place due to selfing, then it is likely that a survey of other populations would turn up alternate alleles at many loci since different alleles tend to become fixed in different populations of selfing plants (Barrett and Shore, 1989).

*A. holboellii* is very different from the nearly monomorphic autogamous taxa described above. Population surveys showed substantial variation in alleles (also see Roy 1993b) and considerable heterozygosity. Analysis of progeny arrays demonstrated that the heterozygosity is

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"fixed" (heterozygous loci remain heterozygous in progeny arrays), suggesting apomixis. However, polyploid selfing could also produce the observed pattern of fixed multilocus heterozygosity (e.g., *Turnera*; Barrett and Shore, 1989), these patterns are heterozygous-like but result from gene duplications and not from true heterozygosity). In autogamous polyploids, crosses among different apparently heterozygous genotypes will segregate yielding different multiple-banded patterns in the progeny (illustrated in Barrett and Shore, 1989). In pseudogamy, crosses among dissimilar genotypes only yield the maternal genotype. Cytology and crossing data rule out polyploid selfing in *A. holboellii.* Of the 12 genotypes examined cytologically, two were diploid (D1 and B11, Table 1, but even these two diploids showed fixed heterozygosity in progeny arrays, a strong indication of apomixis). Crossing experiments showed that pollen was required for seed set in *A. holboellii,* but alleles did not segregate in the progeny, which all bore the maternal genotype, indicating pseudogamous apomixis instead of autogamy. Pseudogamy has also been suggested for Canadian and Greenland populations of *A. holboellii* by Böcher (1951).

*Arabis holboellii* may have a mixed mating system in Colorado. Pseudogamy appears to be the most common mode of reproduction since the majority of plants tested in the crossing experiment (44 out of 47) required pollen for seed initiation, and the progeny from these crosses always had the maternal genotype. However, in my emasculation trials, three mothers out of 23 set a few (2–8 seeds) in the absence of pollen. This small number of fertile emasculated flowers may be the result of pollen contamination from incomplete anther removal and not true seed set by emasculated flowers, or it could represent occasional autonomous apomixis. *A. holboellii* may also occasionally reproduce sexually. Sexual reproduction is suggested by rare occurrences of homozygotes in population samples (B. A. Roy, unpublished data). Genetic variation in *A. holboellii* is probably due to polyphyletic origin from sexual ancestors or from sexual-apomictic crosses. Polyphyletic origin is strongly indicated by differences in chromosome numbers and by the dissimilar banding patterns among genotypes. Sexual-apomictic crosses that create new apomictic genotypes are likely when both breeding systems are present in a population because pollen viability remains high in apomictic *Arabis* (percent stainable pollen, Table 1).

Like *Arabis holboellii,* the allozymes found in *A. hirsuta* are variable at the population level, and progeny arrays revealed fixed heterozygosity, suggesting apomixis. However, I cannot rule out the possibility that these individuals may be selfing polyploids because I did not examine progeny from crosses between known different genotypes. Polyploidy is the norm in *A. hirsuta.* Tetraploid chromosome counts of *A. hirsuta* are common in Europe (Titz, 1972), Canada (Mulligan, 1964), and have also been reported from Gunnison County, Colorado (Rollins, 1941). Tetraploids can be either apomictic or sexual (because the chromosome number is even, meiotic pairing can be normal [Lewis, 1980; Levin, 1983]). Titz (1972) reports that European *A. hirsuta* plants require pollen for seed set. He concludes that *A. hirsuta* is sexual and autogamous because it normally selfs during flowering, has normal meiosis, and he has made successful inter- and intraspecific crosses. My data suggest that the *A. hirsuta* I surveyed are pseudogamous because in this allozymically variable species there was no segregation of alleles in progeny arrays. However, these progeny arrays probably resulted from self pollen since the seeds used were collected from

TABLE 3. Population survey of 19 putative loci of six species of *Arabis.* Allelic frequencies for putative apomictic taxa (*A. gunnisoniana, A. hirsuta, A. holboellii,* and *A. lignifera*) are not given because duplications, overlapping bands, and unbalanced heterozygosity make calculations of gene frequencies problematic (apomictic taxa cannot be crossed to verify loci and numbers of alleles). Instead of allelic frequencies, the frequency of individuals exhibiting a band is given for the fixed heterozygotes. Dashes indicate missing data.

| Locus-allele | | Species (Population) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | DRUM (Gold) $N = 25$ | DRUM (Gothic) $N = 18$ | CRAN (Almont) $N = 25$ | HIR (Gold) $N = 21$ | GUN (Gold) $N = 22$ | LIG (Cement) $N = 17$ | HOLB (Cement) $N = 30$ |
| AAT1 | a | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 |
| | b | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 |
| | c | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 | 0.00 |
| AAT 2 | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| AAT 3 | a | 1.00 | 1.00 | 1.00 | 0.81 | 1.00 | 1.00 | 1.00 |
| | a | 0.00 | 0.00 | 0.00 | 0.19 | 0.00 | 0.00 | 0.00 |
| ACN 1 | a | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 1.00 | 1.00 |
| | b | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | c | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 |
| ACN 2 | a | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 1.00 |
| | b | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 |
| | c | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | d | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 |
| AcPh 1 | a | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| | b | 0.00 | 0.00 | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 |
| | c | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | d | 0.00 | 0.00 | 0.86 | 1.00 | 1.00 | 1.00 | 1.00 |
| | e | 1.00 | 1.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| FE 1 | a | 1.00 | 1.00 | 0.00 | 0.95 | 1.00 | 1.00 | 1.00 |
| | b | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 |
| FE 2 | a | — | — | 1.00 | 0.95 | 0.00 | 0.00 | 0.00 |
| | b | — | — | 0.00 | 0.05 | 0.00 | 0.00 | 1.00 |
| FE 3 | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| IDH 1 | a | 1.00 | 1.00 | 0.00 | 0.04 | 1.00 | 1.00 | 1.00 |
| | b | 0.00 | 0.00 | 1.00 | 0.96 | 0.00 | 0.00 | 0.00 |
| IDH 2 | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | b | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 |
| LAP 1 | a | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 |
| | b | 1.00 | 0.61 | 0.02 | 0.02 | 0.00 | 1.00 | 1.00 |
| | c | 0.00 | 0.39 | 0.98 | 0.00 | 0.00 | 0.00 | 1.00 |
| | d | 0.00 | 0.00 | 0.00 | 0.98 | 1.00 | 1.00 | 0.00 |
| LAP 2 | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| ME 1 | a | 0.61 | 0.55 | 1.00 | 0.95 | 1.00 | 1.00 | 0.87 |
| | b | 0.39 | 0.42 | 0.00 | 0.05 | 0.00 | 0.00 | 0.13 |
| | c | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI 1 | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGI 2 | a | 0.08 | 0.00 | 1.00 | 0.55 | 1.00 | 1.00 | 1.00 |
| | b | 0.80 | 0.81 | 0.00 | 0.45 | 1.00 | 1.00 | 1.00 |
| | c | 0.12 | 0.19 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 |
| 6PGD 2 | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| SKDH 1 | a | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 1.00 | 1.00 |
| | b | 1.00 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | c | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 |
| TPI 1 | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | b | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 |
| TPI 2 | a | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 | 1.00 | 1.00 |
| | b | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 |

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wild plants. So, until I make crosses between known different allozyme genotypes to check for segregation in the progeny, and verify chromosome numbers, the breeding system of these *A. hirsuta* could be either pseudogamous or autogamous.

The fourth breeding system group includes *A. lignifera* and *A. gunnisoniana.* The preponderance of the evidence suggests that both *A. lignifera* and *A. gunnisoniana* are pseudogamous apomicts with little or no genetic variation in their populations. Progeny analysis yielded fixed heterozygotes, and the crossing experiment established that pollen was necessary for seed set, yet there was no evidence of a paternal contribution to the progeny in either species. I found no population-level allelic variation in either *A. lignifera* or *A. gunnisoniana*; however, this result may represent an underestimate of allozyme diversity since only one population per species was surveyed. Cytology provides additional evidence for apomixis in this group. I have only observed diploid numbers of chromosomes in *A. lignifera*; thus the fixed heterozygosity detected with electrophoresis is best explained by apomixis and not by polyploid selfing. *A. gunnisoniana* is polyploid (triploid), but all material examined showed signs of irregular meiosis such as improper pairing (Fig. 5) and the production of dyads instead of tetrads, indicating apomixis and not polyploid selfing.

Three aspects of pseudogamy in *Arabis* are interesting because they suggest that the evolution of pseudogamy may be different or more complex than currently accepted. First, all of the pseudogamous species in this study are self-compatible, and due to the tendency of the anthers to release pollen in the bud, self pollen is the pollen most likely to fertilize the endosperm. Pseudogamous apomicts are thought to be derived from closely related self-incompatible outcrossing species (Gustafsson, 1946–1947; Asker and Jerling, 1992; Mogie, 1992), yet there is little evidence that outcrossing is common in *Arabis* since autogamy and apomixis are the most commonly reported breeding systems (Rollins, 1941; Böcher, 1951, 1954, 1969; Titz, 1972; Vorobik, 1985; Roy and Rieseberg, 1989). As far as I know, self-incompatibility has only been reported in two species of *Arabis, A. aculeolata* and *A. modesta,* which are localized taxa of southwest Oregon and northwest California (Vorobik, 1985). Neither of the self-incompatible species is thought to be closely related to the *Arabis* discussed here (Rollins, 1941; Vorobik, 1985).

The second interesting point about pseudogamy in *Arabis* is that the maternal plants were not always particular about the source of the pollen that fertilized the endosperm. For example, *A. holboellii* endosperm could be fertilized either by self-pollen, or by pollen from different genotypes within the same species, and *A. gunnisoniana* set fruit when pollen from a different species, *A. holboellii,* was used in the cross. This last result suggests either: (1) the pollen is not fertilizing the endosperm but is instead triggering development in some other way, or, (2) that pollen of *A. holboellii* is similar enough to *A. gunnisoniana* to effect fertilization of the endosperm. Interestingly, a similar pattern of pollen compatibility is found in blackberries (*Rubus*). Nybom (1989) reports that pseudogamous blackberries set seed after the application of self pollen, interspecific pollen, or intraspecific pollen as long as the pollen:ovule ratios are the same.

The final oddity about pseudogamy in *Arabis* is that apomixis in plants is strongly associated with polyploidy, and diploid examples of gametophytic apomixis (gametophyte present but with an unreduced chromosome complement, as is the case in *Arabis* [Böcher, 1951]) are quite rare (Gustafsson, 1946–1947; Stebbins, 1950; Nygren, 1954; Asker, 1980; Grant, 1981; Bierzychudek, 1985). In *Arabis,* however, it may be fairly common to find diploid apomixis. Here I have presented evidence that some Colorado populations of *Arabis lignifera* and at least two genotypes of *A. holboellii* (D1 and B11, Table 1; Fig. 3) are diploid apomicts. Diploid apomixis has also been reported by Böcher, on the basis of cytology and embryology, from several arctic collections of *Arabis holboellii* (Böcher, 1951, 1969) and from *A. microphylla* (Böcher, 1969).

In this study I have illustrated the value of using several different tools to evaluate breeding systems. The exclusion of insects indicated when pollen was necessary for seed set, but did not allow me to differentiate between pseudogamy and autogamy. The electrophoresis data gave information on the relative degree of selfing (as evidenced by homozygosity) and apomixis (fixed heterozygosity), but I could not differentiate between selfing and pseudogamy in polyploid species with no allozyme variation. Crosses between dissimilar genotypes confirmed pseudogamy when they always yielded progeny bearing the maternal genotype. Finally, the cytological data allowed me to differentiate between diploids and polyploids, and directly confirmed irregular meiosis in apomicts.

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