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CHLOROPLAST DNA AND ISOZYME DIVERSITY IN TWO MIMULUS SPECIES (SCROPHULARIACEAE) WITH CONTRASTING MATING SYSTEMS[1]

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Levels of cpDNA and isozyme diversity were contrasted between the mixed-mating *M. guttatus* and its highly selfing congener *M. micranthus* (Scrophulariaceae). Compared to *M. micranthus, M. guttatus* has two to four times higher diversity for both cpDNA and isozyme variation on a species-wide level. The selfing *M. micranthus* also has 1.5 to three times lower within-population allozyme variation and a greater proportion of its variation distributed among rather than within populations. Chloroplast DNA is here inferred to be uniparentally inherited. Thus the mating system has no effect on the transmission of the chloroplast genome. Since both cpDNA and isozyme variation are similarly reduced in *M. micranthus,* factors other than the mating system are hypothesized to be responsible for the observed decrease in species-wide genetic variation in *M. micranthus.* A recent origin of *M. micranthus* from a limited number of *M. guttatus* populations is suggested. Consequently, molecular variation is reduced in *M. micranthus* due to a bottleneck effect. These data generally demonstrate that levels of cpDNA variation may be high enough in some species to infer evolutionary processes below the species level.

The mating system, or the degree that a plant selfs vs. outcrosses, plays an important role in determining patterns of genetic variation for nuclear, biparentally inherited genes (Allard, Jain, and Workman, 1967; Wright, 1969; Jain, 1976). Population levels of variability for allozymes are generally lower for selfers, and a greater proportion of variation is distributed among populations for selfers as opposed to outcrossers (Hamrick, Linhart, and Mitton, 1979; Loveless and Hamrick, 1984). Lower levels of within-population variation are expected for selfers since the effective population size, or size of the panmictic unit (Wright, 1943, 1946), will be correlated to pollen dispersal distances, which are reduced in selfers (Crawford, 1984). Low effective population sizes increase the probability of random drift. Consequently, selfers are expected to have increased homozygosity within populations and greater differentiation between populations (Falconer, 1981). Although selfing primarily affects the apportionment of genetic diversity and not the total species-wide level of diversity (Falconer, 1981), selfers nevertheless are frequently observed to have lower overall genetic variation compared to outcrossers (Hamrick and Godt, 1989). In contrast, because cpDNA is uniparentally inherited, its transmission should be completely unaffected by mating system (Palmer, 1987). Here we demonstrate that we can use levels of cpDNA variation to quantify the role mating system plays in determining levels of variation of nuclear, biparentally inherited loci.

In plants it is generally thought that cpDNA may evolve at rates sufficient to result in detectable differences among species within a genus (Palmer, 1987). Of the few studies that have attempted to quantify the amount of cpDNA variation within a species, most have observed little variation (e.g., Clegg, Brown, and Whitfield, 1984; Clegg, Rawson, and Thomas, 1984; Banks and Birky, 1985; Clegg, 1987), while at least several (Soltis, Soltis, and Ness, 1989; Lavin, Mathews, and Hughes, 1991) have observed more variation. Further studies of cpDNA variation are needed, both to determine factors governing its diversity and variation and to examine the utility of cpDNA variation for inference of evolutionary processes below the species level.

The objective of this study was to determine levels of cpDNA and allozyme variation in two closely related species of *Mimulus,* the mixed-mating *M. guttatus* and the highly selfing *M. micranthus.* By contrasting levels of cpDNA and isozyme variation between *M. guttatus* and *M. micranthus,* we hoped to determine the role of mating system in partitioning nuclear genetic variation at the species and population levels.

MATERIALS AND METHODS

Mimulus guttatus DC (Scrophulariaceae) is an annual to perennial herb found along western North America from Alaska to Mexico and eastward to the Rockies. It has a mixed-mating system with populations selfing at rates from 25% to 60% (mean = 48%, Ritland, 1990). In contrast, *M. micranthus* is a highly selfing annual with a selfing rate of 84% (Ritland and Ritland, 1989), and is restricted to the coastal range of central California. The floral architecture of these two species differs dramatically (see Fig. 1 in Ritland and Ritland, 1989), and corresponds to their mating system: large-flowered *M. guttatus* has a high pollen-ovule ratio, and is herkogamous, whereas *M. micranthus* lacks herkogamy, is autogamous, and invests relatively little in floral display or pollen production (Ritland and Ritland, 1989). Because 1) some taxonomists have termed *M. micranthus* a subspecies of *M. guttatus* (Campbell, 1950), 2) *M. guttatus* has a much greater range than *M. micranthus,* and 3) the two species have contrasting mating systems, we a priori considered *M. guttatus* and *M. micranthus* as a progenitor-derivative pair.

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TABLE 1. *Pattern of chloroplast DNA variation across eight populations of* M. guttatus *and five populations of* M. micranthus. *Plus and minus signs indicate presence or absence of a restriction site, respectively*[a]

	*Xba*I	*Cla*I	*BamH*I	*Dra*I	*EcoR*I	*Ban*I	*Stu*I	*Kpn*I
M. guttatus								
202	+	−	−	−	+	−	+	−
118	+	+	−	−	+	−	+	−
201	+	+	−	−	+	+**	+	−
124	+	+	−	−	−*	−	+	+
125	+	+	−	−	−*	−	+	+
104	−	+	−	−	+	−	+	+
208	−	+	−	−	+	−	+	+
101	−	+	−	−	+	−	−	+
M. micranthus								
306	−	+	+	−	+	−	+	+
302	−	+	+	−	+	−	+	+
301	−	+	+	−	+	−	+	+
305	−	+	+	−	+	−	+	+
307	−	+	+	+	+	−	+	+

[a] * Putative deletion; ** putative insertion.

To determine levels of cpDNA variation, we sampled one individual plant from each of eight and five populations in *M. guttatus* and *M. micranthus,* respectively. Localities are given in Appendix 1. Of the eight *M. guttatus* populations, six were within 200 km of San Francisco (within the Coast Range) and two populations (from Tuolumne Co.) were east of the Central Valley (in the foothills of the Sierra). Approximately 120 selfed progeny per sampled plant were grown in a growth chamber, and 50–100 g of leaves was harvested from each group of selfed progeny. Chloroplast DNA was isolated and purified following the method of Hosaka and Hanneman (1987). Purified cpDNA (1–2 mg) was digested with restriction enzymes (Pharmacia) according to instructions. DNA fragments were separated on 0.7% agarose gels (Palmer, 1986), run for 13–16 cm, and visualized with ethidium bromide observed under UV illumination. The following 27 enzymes were used in single digests: *Ava*II, *BamH*I, *Ban*I, *Bcl*I, *Bgl*II, *Cla*I, *Dra*I, *EcoR*I, *EcoR*IV, *Hinc*II, *Hind*III, *Hpa*I, *Kpn*I, *Mlv*I, *Nhe*I, *Nrv*I, *Pst*I, *Pvu*I, *Sac*I, *Sac*II, *Sal*I, *Sca*I, *Sma*I, *Sph*I, *Stu*I, *Xba*I, and *Xho*I.

For each species, the proportion of polymorphic nucleotide sites (**p**) (here, the probability that a given nucleotide site is polymorphic in a sample of cpDNA molecules) was estimated using the method of Engels (1981). This estimator of **p** assumes that a given restriction site contains no more than one nucleotide polymorphism and is most appropriate for mutations due to nucleotide substitution resulting in the gain or loss of restriction sites. Chloroplast DNA diversity was also estimated using the statistic **h** (Nei and Tajima, 1981), which quantifies total diversity, including deletions and insertions, and here measures the probability that two randomly chosen cpDNA molecules will be different. Since levels of cpDNA diversity are low compared to other genomes (Clegg, 1987; Birkey, 1989) we focused our effort on quantifying cpDNA diversity by sampling more populations in lieu of sampling within populations. Thus no estimate of intrapopulation cpDNA variation was possible since only one cpDNA genome was sampled per population.

Thirteen isozyme loci known to be polymorphic in *Mimulus* (Ritland and Ganders, 1987; Vickery and Wullstein, 1987; Ritland, 1989; Ritland and Ritland, 1989) were surveyed for their degree of polymorphism in the same populations sampled for cpDNA. These enzymes were Dia 1, Dia 2, Est 1, Est 2, Idh 1, Mdh 1, Mdh 2, Pgm, 6Pgd 1, PGD 2, Pgi, Tpi 1, and Tpi 2. Gel buffers and staining conditions are given in Ritland and Ganders (1987). Enzymes were extracted from a minimum of 30 individuals per population (three seedlings per maternal family from a minimum of ten field-collected plants).

Species-wide estimates of diversity were based on the proportion of polymorphic loci ($\mathbf{P_s}$) and average number of alleles/locus ($\mathbf{A_s}$). Genetic diversity ($\mathbf{H_{es}}$), the expected heterozygosity when populations are pooled and allowed to mate randomly, was calculated for each locus where $\mathbf{H_{es}} = 1 - \Sigma\, p_i^2$ and p_i is the mean frequency of the i^{th} allele for each species. The effective number of alleles ($\mathbf{A_{es}}$) was also determined where $\mathbf{A_{es}} = 1/(1 - \mathbf{H_{es}})$. Mean genetic diversity was calculated by averaging the values across loci. Levels of intrapopulation diversity were quantified by determining the percentage of polymorphic loci ($\mathbf{P_p}$), and average number of alleles per locus ($\mathbf{A_p}$). Within-population levels of genetic diversity were also measured as $\mathbf{H_{ep}}$, the expected heterozygosity if individuals in a population are allowed to randomly mate, where $\mathbf{H_{ep}} = 1 - \Sigma\, p_i^2$ and p_i is the mean frequency of the i^{th} allele for each population. The effective number of alleles at the population level ($\mathbf{A_{ep}}$) was also determined where $\mathbf{A_{ep}} = 1/(1 - \mathbf{H_{ep}})$. Mean genetic diversity at the population level was calculated by averaging the values across loci and populations. The proportion of variation distributed within vs. between populations was measured using Nei's diversity statistics, i.e., $\mathbf{G_{ST}}$ (Nei, 1975).

To control for number of populations sampled and geographic range surveyed for each species, measures of variation were determined for *M. guttatus* by limiting the sample to five of the six populations along the coastal range, because five populations over this area were sampled for *M. micranthus.* The different measures of genetic variation for *M. guttatus* were determined as the mean of the six different ways of sampling five populations from six.

RESULTS

Although 19 of 27 restriction digests revealed no cpDNA polymorphisms (Fig. 1A), eight restriction enzymes did reveal cpDNA polymorphisms either within or among *M. guttatus* or *M. micranthus*: *BamH*I (Fig. 1B), *Ban*I, *Cla*I, *Dra*I, *EcoR*I (Fig. 1C), *Kpn*I, *Stu*I, and *Xba*I (Fig. 1D). Table 1 summarizes the pattern of cpDNA variation for all 13 populations of both taxa, and gels representing a subset of the observed polymorphisms are shown in Fig. 1. Two mutations were unique to *M. micranthus, BamH*I (Fig. 1B) and *Dra*I, of which the latter was limited to one population. Aside from this one mutation, no other cpDNA polymorphisms were found in *M. micranthus.* By contrast, *M. guttatus* had six polymorphisms, three (*Ban*I, *Cla*I, and *Stu*I) unique to each of three populations, one (*EcoR*I, Fig. 1C) shared by two populations, and two (*Kpn*I and *Xba*I, Fig. 1D) shared by two different groups of three populations. Two of five mutations in *M. guttatus* were not easily interpreted as point mutations. One such mutation appears to be a deletion within a region flanked

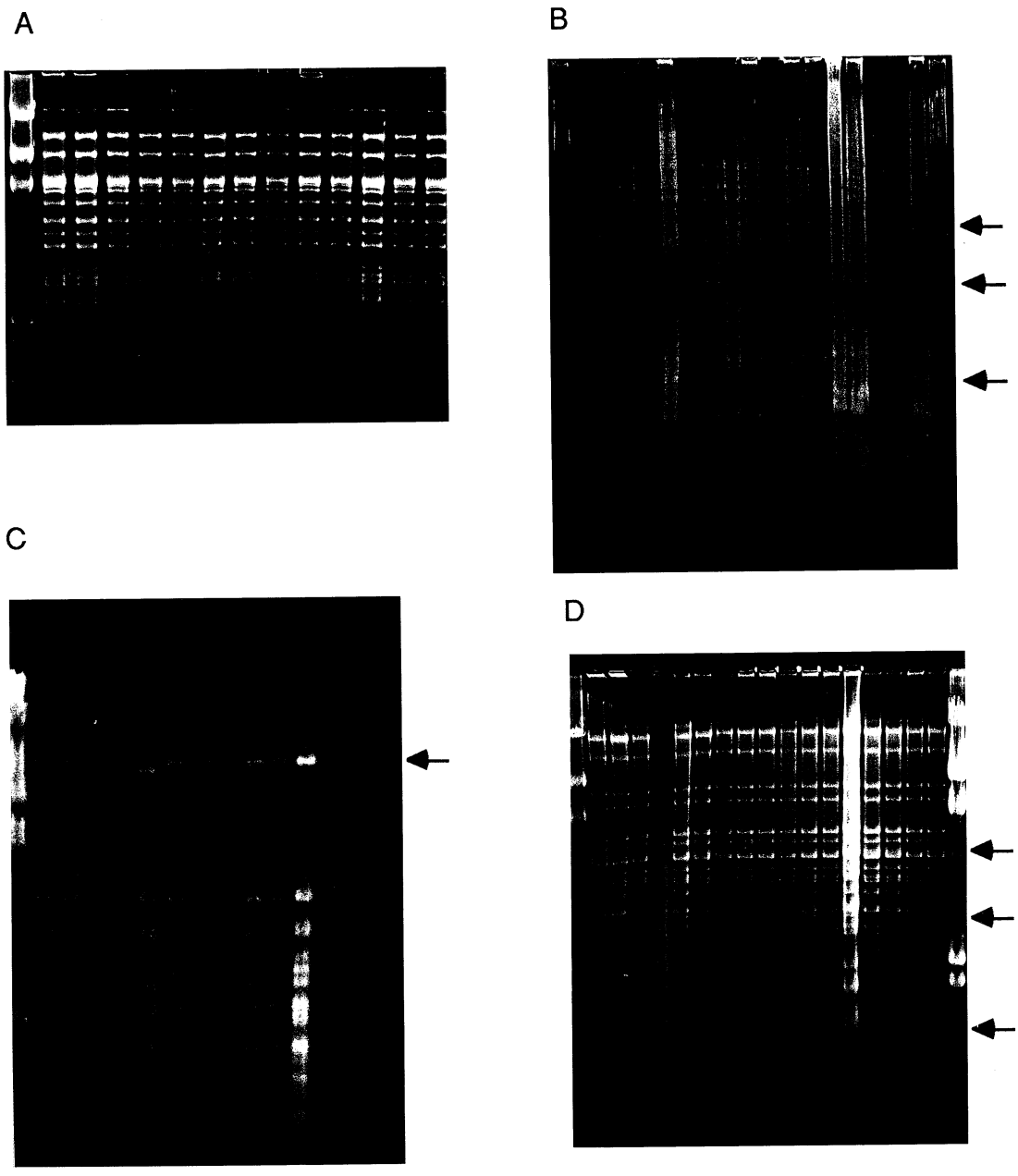

Fig. 1. Typical purified cpDNA patterns showing no variation in *Mimulus* following restriction by A) *Bcl*I, and variation following restriction by B) *BamH*I, C) *EcoR*I, and D) *Xba*I. Arrows indicate locations on gel where polymorphisms were observed. First lane (A and C) and first and last lanes (B and D) and λ restricted with *Hind*III.

by two restriction sites for *EcoR*I (Fig. 1C). The second such mutation appears to be an insertion within a region flanked by two restriction sites for *Ban*I. Because these putative insertion/deletion mutants were not detected when other endonucleases were used, they may be point mutations with overlapping bands as observed by Soltis, Soltis, and Ness (1989). Alternatively, the smaller fragments associated with the loss of the larger molecular weight bands may have gone undetected.

If we include the insertions and deletions, an upper estimate of the proportion of cpDNA nucleotides polymorphic (**p**) in *M. guttatus* is 0.00228 (0.00093 SE) while that for *M. micranthus* is 0.00057 (0.00057 SE) (Table 3). These differences are marginally significant (there is only slight overlap of the means with two standard errors), although we note that it is difficult to establish statistical significance with the relatively low number of mutational events. Overall, it appears that *M. guttatus* has about four times the chloroplast polymorphism of *M. micranthus.* Chloroplast diversity (**h**) was also greater in *M. guttatus* vs. *M. micranthus* (0.92 ± 0.07 SE vs. 0.40 ± 0.24 SE, respectively). Here the means are clearly significantly different (no overlap of the means with two standard errors), with cpDNA diversity higher in *M. guttatus.*

When sampling was confined to only those populations of *M. guttatus* that overlapped in range with *M. micranthus,* **p** was still almost three times larger for *M. guttatus* (0.00138) and **h** was essentially unchanged. Note that the

TABLE 2. *Allele frequencies at all loci surveyed in* M. micranthus *and* M. guttatus. *Population numbers are collection numbers referenced in Appendix 1*

Locus	Allele	Population												
		M. guttatus								*M. micranthus*				
		101	104	118	124	125	202	208	201	301	302	305	306	307
Est-1	1	0.971	0.741	0.917	0.017	0.400	0.071	0.770	0.038	0.031	0.026	0.0	0.0	0.0
	2	0.029	0.017	0.033	0.466	0.400	0.543	0.148	0.442	0.967	0.974	1.0	0.886	1.0
	3	0.0	0.0	0.0	0.241	0.029	0.043	0.0	0.231	0.0	0.0	0.0	0.0	0.0
	4	0.0	0.0	0.0	0.155	0.100	0.014	0.0	0.212	0.0	0.0	0.0	0.0	0.0
	5	0.0	0.086	0.050	0.121	0.029	0.229	0.0	0.038	0.0	0.0	0.0	0.114	0.0
	6	0.0	0.017	0.0	0.0	0.029	0.0	0.082	0.0	0.0	0.0	0.0	0.0	0.0
	7	0.0	0.034	0.0	0.0	0.014	0.100	0.0	0.038	0.0	0.0	0.0	0.0	0.0
	8	0.0	0.103	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Est-2	1	0.984	0.786	0.867	0.900	0.971	0.615	0.667	0.167	1.0	1.0	1.0	1.0	1.0
	2	0.016	0.214	0.133	0.100	0.029	0.385	0.333	0.797	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.037	0.0	0.0	0.0	0.0	0.0
Dia-1	1	0.033	0.129	0.432	0.276	0.304	0.516	0.412	0.867	0.026	0.0	0.0	0.100	0.0
	2	0.967	0.871	0.568	0.724	0.696	0.484	0.588	0.133	0.974	1.0	1.0	0.900	1.0
Dia-2	1	0.929	0.814	0.105	0.453	0.413	0.773	0.768	0.575	1.0	1.0	1.0	0.882	1.0
	2	0.014	0.0	0.553	0.297	0.175	0.0	0.071	0.273	0.0	0.0	0.0	0.0	0.0
	3	0.043	0.186	0.342	0.172	0.286	0.227	0.125	0.152	0.0	0.0	0.0	0.118	0.0
	4	0.014	0.0	0.0	0.078	0.127	0.0	0.036	0.0	0.0	0.0	0.0	0.0	0.0
Idh	1	0.548	0.500	0.441	0.344	0.643	0.585	0.921	0.545	1.0	0.542	1.0	0.656	0.0
	2	0.452	0.500	0.559	0.656	0.357	0.132	0.079	0.236	0.0	0.457	0.0	0.344	1.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.200	0.0	0.0	0.0	0.0	0.0
	4	0.0	0.0	0.0	0.0	0.0	0.283	0.0	0.018	0.0	0.0	0.0	0.0	0.0
Mdh-1	1	1.0	0.732	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	0.0	0.268	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mdh-2	1	0.043	0.507	0.250	0.157	0.457	0.219	0.200	0.114	0.0	0.0	0.01	0.0	0.0
	2	0.957	0.493	0.750	0.843	0.543	0.781	0.800	0.886	1.0	1.0	0.99	1.0	1.0
Tpi-1	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tpi-2	1	0.0	0.0	0.0	0.0	0.0	0.176	0.359	0.171	0.0	0.0	0.0	0.0	0.0
	2	1.0	1.0	1.0	1.0	1.0	0.824	0.641	0.829	1.0	1.0	1.0	1.0	1.0
Pgi	1	0.574	0.492	0.679	0.569	0.426	0.343	0.167	0.185	0.051	0.371	0.250	0.271	0.424
	2	0.015	0.190	0.321	0.345	0.500	0.567	0.652	0.738	0.949	0.571	0.176	0.729	0.322
	3	0.0	0.0	0.0	0.086	0.074	0.0	0.106	0.0	0.0	0.057	0.0	0.0	0.0
	4	0.412	0.317	0.0	0.0	0.0	0.090	0.045	0.077	0.0	0.0	0.574	0.0	0.254
	5	0.0	0.0	0.0	0.0	0.0	0.0	0.030	0.0	0.0	0.0	0.0	0.0	0.0
6Pgd-1	1	1.0	1.0	1.0	0.986	0.824	1.0	0.986	1.0	1.0	1.0	1.0	1.0	1.0
	2	0.0	0.0	0.0	0.014	0.074	0.0	0.014	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.103	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6Pgd-2	1	0.057	0.029	0.0	0.294	0.788	1.0	0.970	0.808	1.0	1.0	1.0	0.914	1.0
	2	0.943	0.971	1.0	0.691	0.150	0.0	0.030	0.141	0.0	0.0	0.0	0.086	0.0
	3	0.0	0.0	0.0	0.0	0.038	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	4	0.0	0.0	0.0	0.015	0.025	0.0	0.0	0.051	0.0	0.0	0.0	0.0	0.0
Pgm	1	0.0	0.0	0.0	0.014	0.164	0.0	0.065	0.015	0.0	0.0	0.0	0.0	0.0
	2	1.0	1.0	1.0	0.757	0.837	0.957	0.742	0.727	1.0	1.0	1.0	1.0	1.0
	3	0.0	0.0	0.0	0.214	0.0	0.0	0.194	0.258	0.0	0.0	0.0	0.0	0.0
	4	0.0	0.0	0.0	0.014	0.0	0.043	0.0	0.0	0.0	0.0	0.0	0.0	0.0

proposed *EcoR*I length variant was not included in this sample because the mutation was found in populations outside the range of *M. micranthus*. When *Ban*I was eliminated as a variant, **p** was still approximately twofold higher in *M. guttatus* (0.00109).

Similar extremes of species-wide genetic variation were observed for the isozyme data (Tables 2, 3). In addition to *M. guttatus* having more polymorphic loci (92% vs. 54% of 13 loci), it also had a greater mean number of alleles per locus (3.4 vs. 1.8) and a higher effective number of alleles (1.8 vs. 1.2) compared to *M. micranthus* (Table 3). Total genetic diversity based on isozymes, $\mathbf{H}_{es}$, was 0.357 for *M. guttatus*, while that for *M. micranthus* was over three times less, at 0.097. The average genetic distance among populations of *M. guttatus* was 3.6 times higher than among populations of *M. micranthus* (0.157 vs. 0.044).

The average number of polymorphic isozyme loci ($\mathbf{P}_p$) per population was approximately threefold greater in *M. guttatus* than in *M. micranthus* (0.71 vs. 0.23, respectively, Table 3), and *M. guttatus* had 2.2 alleles per locus vs. 1.3 for *M. micranthus* and a greater effective average number of alleles/locus (1.4 vs. 1.1) averaged across populations. The outcrossing *M. guttatus* had higher heterozygosity within populations ($\mathbf{H}_{ep}$ = 0.264) and less divergence among populations ($\mathbf{G}_{ST}$ = 0.262), compared to *M. micranthus* ($\mathbf{H}_S$ = 0.064, $\mathbf{G}_{ST}$ = 0.343). Except for genetic distance the mean differences between the two species for measures of allozyme diversity did not overlap with two standard errors and thus are considered to be significantly different from each other. None of the estimates of genetic diversity based on allozyme variability changed when only those populations of *M. guttatus* that overlapped in range with *M. micranthus* were used.

TABLE 3. *Summary of levels of diversity differences between* M. guttatus *and* M. micranthus. *Standard errors are in parentheses*

Parameter	*M. guttatus* (mix mating)		*M. micranthus* (selfer)		Ratio of *M. guttatus* / *M. micranthus*
Species-wide diversity (cpDNA)					
Probability that a cpDNA nucleotide site is polymorphic (**p**)	0.00228	(0.00093)	0.00057	(0.00057)	4.0
	0.00138[a]				2.4
Probability two randomly chosen cpDNA molecules are different (**h**)	0.92	(0.07)	0.40	(0.24)	2.3
Species wide diversity (allozymes)					
Percentage of polymorphic loci ($\mathbf{P_s}$)	92.0	(2.0)	54.0	(4.0)	1.7
Average number of alleles/locus ($\mathbf{A_s}$)	3.4	(0.6)	1.8	(0.3)	1.9
Effective number of alleles/locus ($\mathbf{A_{es}}$)	1.8	(0.2)	1.2	(0.1)	1.5
Genetic diversity ($\mathbf{H_{es}}$)	0.357	(0.063)	0.097	(0.054)	3.6
Average genetic distance among populations	0.157	(0.078)	0.044	(0.037)	3.6
Population levels of diversity (allozymes)					
Percentage of polymorphic loci ($\mathbf{P_p}$)	71.0	(10.0)	23.0	(8.0)	3.1
Average number of alleles/locus ($\mathbf{A_p}$)	2.2	(0.1)	1.3	(0.1)	1.7
Effective number of alleles/locus ($\mathbf{A_{ep}}$)	1.4	(0.10)	1.1	(0.1)	1.1
Genetic diversity ($\mathbf{H_{ep}}$)	0.235	(0.050)	0.064	(0.035)	3.7
Proportion of diversity within populations ($\mathbf{G_{ST}}$)	0.262		0.343		0.8

[a] Estimated using only the six populations of *M. guttatus* that overlap in range with *M. micranthus*. All other parameters were unaffected by restricting the sample size.

DISCUSSION

Of eight cpDNA genomes sampled in the outcrossing *M. guttatus,* six had distinct or unique restriction profiles due to either point substitutions, insertions, or deletions. However, only one of five *M. micranthus* cpDNA genomes differed. These contrasting patterns were reflected in the two to four times higher estimates of cpDNA diversity in *M. guttatus* vs. *M. micranthus* (Table 3). When geographic range was taken into account, cpDNA diversity was still two to three times higher in *M. guttatus* than *M. micranthus.* The relative levels of species-wide isozyme diversity in *M. guttatus* vs. *M. micranthus* were similar to patterns of cpDNA variation with *M. guttatus* showing 1.5 to four times more isozyme diversity than *M. micranthus* (Table 3).

Inbreeding per se is not expected to result in the loss of genetic diversity within a species, but will change the distribution of genetic variation (Falconer, 1981). Highly self-fertilizing species often have lower heterozygosity within populations and a greater proportion of their genetic variation distributed among populations compared to outcrossers for allozymes (Levin, 1978; Brown and Jain, 1979; Ellstrand and Levin, 1980; Layton and Ganders, 1984; Loveless and Hamrick, 1984). The reduced diversity is caused by both bottlenecking of population size due to the colonization habit of selfers, and by a reduced effective population size (at most by half) due to selfing and restriction of gene flow to seed dispersal (Hillel, Feldman, and Simchen, 1973). Thus it is of little surprise that *M. micranthus* has both lower levels of population diversity and a higher proportion of allozyme variation distributed among populations compared to *M. guttatus.* However, species-wide diversity is not expected to be affected by mating system. In a survey of 473 species Hamrick and Godt (1989) observed that selfers generally had 30%–50% less species-wide diversity than outcrossers, although there was no difference between selfers and species that were mixed-mating. Why, then, is the overall species-wide genetic diversity of selfers lower than outcrossers, and in particular, why does *M. micranthus* have both lower cpDNA and allozyme species-wide variation than *M. guttatus*?

Selfers have lower between-population gene flow (Hamrick and Godt, 1989), thus new mutations in selfers may not spread before populations go extinct (Hamrick, personal communication). This may explain some of the differences between selfers and outcrossers for nuclear genes, but gene flow for cpDNA is likely restricted to seeds regardless of mating system. We have proposed that joint measurements of cpDNA and isozyme diversity allow the relative role of selfing in patterning genetic variation to be elucidated. Since the differences in diversity between *M. guttatus* and *M. micranthus* for cpDNA vs. isozymes are about the same (two to four times), we hypothesize that inbreeding due to selfing is not responsible for the differences of diversity between these taxa with contrasting mating systems. Instead, the uniformity of differences in levels of cpDNA and isozyme variation may provide insight into the timing and mechanism of the origin of this selfing taxon.

The mean genetic distance among all members of *M. micranthus* is approximately four times lower than among *M. guttatus* populations, 0.044 vs. 0.157, respectively. Given the small genetic distance among populations of *M. micranthus* relative to *M. guttatus,* we assume that the mutation at the *BamH*I site shared by all *M. micranthus* populations reflects the progenitor-derivative relationship between *M. guttatus* and *M. micranthus* and suggests that *M. micranthus* is monophyletic. The numbers of polymorphic loci and alleles per locus are especially sensitive to population bottlenecks (Leberg, 1992). Thus, for both cpDNA and isozymes, the amount of variation within *M. micranthus* probably reflects the variation found with a single or a few ancestral *M. guttatus* populations with no subsequent increase in variation due to mutation. If *M. micranthus* evolved recently, then the number of

cpDNA mutations within the species would be expected to be low due to the conservative evolution of the cpDNA molecule (Palmer, 1987). Note that *M. guttatus* populations 104 and 208 differ from *M. micranthus* populations 301, 302, 305, and 306 by only one restriction site mutation (*BamHI*). A recent origin of *M. micranthus* is also supported by the relatively low Nei's genetic distance (0.041) between the most similar populations of *M. guttatus* and *M. micranthus,* which is equivalent to the mean genetic distance among *M. micranthus* populations (analysis not shown). Additional evidence for a recent origin is the absence of sterility barriers and complete cross fertility between the two species (Fenster and Ritland, unpublished data). Although hybrid zones between the two are commonly observed in the field, the earlier flowering of *M. micranthus* relative to *M. guttatus* (personal observation) may maintain the integrity of the two species.

The genetic data are also consistent with the patterns of progenitor-derivative species pairs outlined by Gottlieb (1973, 1977, 1981). *Mimulus micranthus* contains only a subset of the allelic variation found within *M. guttatus,* and for all loci the presumed derivative *M. micranthus* is either fixed or polymorphic for the most common alleles of *M. guttatus.* The progenitor-derivative relationship has been documented for *Cirsium* (Loveless and Hamrick, 1988) and for other examples cited therein. In each case the derivative species is less variable and monomorphic at many of the homologous loci. In this context, *M. micranthus* having two unique cpDNA mutations is surprising compared to the uniformity across isozyme loci where *M. micranthus* has only a subset of isozyme variation and no unique alleles. The effective population size for cpDNA is one quarter that of nuclear biparentally inherited DNA because it is haploid and likely uniparentally inherited. Thus cpDNA must be more prone to drift than isozymes (given variation for both is neutral). Unique cpDNA mutations present in *M. micranthus* may therefore be the result of drift or lineage sorting (Avise et al., 1987; Avise, 1989) where identical mutations in *M. guttatus* have been lost due to random extinction events. Many of the species within the genus, including *M. micranthus* and *M. guttatus,* can freely cross with one another (Vickery, 1978; Macnair and Cumbes, 1989; Fenster and Ritland, unpublished data). Therefore introgression may prevent the fixation of alternative nuclear alleles, but the uniparental inheritance of cpDNA may allow the maintenance of separate lineages of cpDNA mutations.

Could the restricted range of *M. micranthus* have confounded our investigation of the role of mating system on genetic diversity? Geographically restricted species have, on average, 1.5 to two times lower species-wide diversity than species with wide-spread distributions, and this difference may be due to smaller population sizes (Hamrick and Godt, 1989). Thus more narrowly distributed species are more prone to drift and bottlenecks with a consequent reduction of genetic diversity. However, when geographic range was held constant, narrowly endemic mix-mating species had an approximate threefold higher level of species-wide isozyme diversity than narrowly endemic selfers (Godt, Hamrick, and Sherman-Broyles, unpublished data). This case roughly corresponds to the situation where we restricted our survey to only those populations of *M. guttatus* that overlapped in range with *M. micranthus.* Therefore, the restricted range of *M. micranthus* does not appear to be responsible for its limited diversity relative to *M. guttatus.* In addition, the limited range of *M. micranthus* should not have an effect on its relatedness with *M. guttatus,* in terms of the alleles present at the homologous loci. Thus a hypothesis of recent origin is not confounded by the limited geographic range of *M. micranthus.*

The overall species genetic diversity will place constraints on the amounts of genetic variation found within populations. In Hamrick's and Godt's (1989) survey genetic diversity was highly correlated between species and population levels. If selfers commonly represent recently derived taxa (Stebbins, 1974; but see Barrett and Shore, 1987; Olmstead, 1989), then lower intrapopulation levels of gene diversity may be due to bottleneck effects associated with the recent formation of selfing species. The evolution of selfing taxa should further compound a bottlenecking effect since the trait is directly associated with the transmission of nuclear genes. Thus, a bottleneck associated with cladogenesis of a selfing taxon will be further increased in effect by the evolution of lower outcrossing rates. Chloroplast DNA variation may be similarly reduced during the evolution of a selfing taxa if one or a few lines of selfing lineages ultimately give rise to the new selfing species. Thus intrapopulation measures of genetic diversity may only be indirectly associated with mating system. Contemporary levels of population genetic variation also may be lower in selfing species if populations are founded by one or a few seeds from a single source population. Colonizing seed from selfing populations is more likely to be homozygous than colonizing seed from outcrossing populations (Layton and Gander, 1984). However, this compounding effect of founders having lower genetic variation in selfers cannot be responsible for species-wide differences in levels of cpDNA diversity.

Although *M. micranthus* has limited amounts of allozyme and cpDNA variation, it does appear to harbor significant and equivalent amounts of genetic variation for floral (corolla tube length, corolla width, pistil length, style length, stamen level, and date of first flower) and vegetative (specific leaf weight, and leaf photosynthetic characters) traits at the species-wide level (Carr and Fenster, unpublished data; Fenster, Forseth, and Carr, unpublished data, respectively). Parallel results have been observed in congeneric comparisons between selfers and outcrossers, e.g., *Phlox* (Clay and Levin, 1989) and *Triticum* (Hillel, Feldman, and Simchen, 1973). There is no reason to believe that patterns of allozyme variation should be concordant with quantitative genetic variation (Lewontin, 1984). Discrepancies could result from stabilizing selection (Allard, Jain, and Workman, 1967), heterozygote advantage (Hillel, Feldman, and Simchen, 1973), or higher mutation rates (Lynch, 1988) associated with polygenic traits. Since the molecular data support the notion of a recent bottleneck in the evolution of *M. micranthus,* we hypothesize that the relatively increased levels of quantitative genetic variation compared to the molecular variation described here for *M. micranthus* reflect higher mutation rates of polygenic traits. Thus the bottleneck associated with the origin of selfing species may have no long-term effect on quantitative variation. Consequently there will be discordance between the pattern of neutral

molecular and morphological (quantitative) variation. Species that have recently been exposed to bottlenecks associated with long-distance colonization events, e.g., *Musca autumnalis* (Bryant, 1984, and examples cited within), often show a correlation between electrophoretic and morphometric variability, thus underscoring that given enough time, polygenic mutation rates may restore quantitative variability.

Chloroplast DNA restriction fragment length polymorphisms have been used to sort out generic (Systma and Gottlieb, 1986) and even species relationships (Palmer and Zamir, 1982; Erickson, Straus, and Beversdorf, 1983; Systma and Schaal, 1985; Systma and Gottlieb, 1986, among others). However, few studies have observed sufficient cpDNA variation within species to allow evolutionary inferences at the population level (Clegg, 1987; Birkey, 1989; but see Soltis, Soltis, and Ness, 1989; Lavin, Mathews, and Hughes, 1991). For example, Banks and Birky (1985) found that of 100 *Lupinus texensis* plants examined among 21 populations, only three variant forms were found. They estimated **p** as 0.00124, which is almost half that found in *M. guttatus* among only eight populations. Higher levels of among-population variation for cpDNA, comparable to that found for *M. guttatus* here, were observed in *Heuchera micrantha* (Soltis, Soltis, and Ness, 1989). They observed 17 mutations (including three length mutations) among 28 populations, giving an estimate of **h** equal to 0.94.

This study has documented a relatively high amount of cpDNA variation within *Mimulus*. Perhaps the levels of cpDNA variation observed here as well as in *Heuchera* are outcomes of common evolutionary processes. Both genera likely have had species with populations persisting for long periods of time in allopatry, reflecting a general trend for high endemism for plant taxa west of the Rockies (Ornduff, 1974). Secondary contact probably has promoted the widespread introgression of nuclear genes, but because of the uniparental inheritance of cpDNA, many different cpDNA lineages remain extant (Harrison and Rand, 1989). The results presented here suggest that levels of cpDNA variation may be high enough in some species to infer evolutionary processes below the species level.

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APPENDIX 1. *Mimulus populations surveyed for cpDNA and isozyme variation*

M. guttatus

101: 1 m S of Pine Flat Road, off Empire Grade, Santa Cruz Co. (28 May 1986).
104: 20202 Skyline Blvd, San Mateo Co. (19 May 1986).
118: Near Pt. Reyes lighthouse at crossroad leading to Chimney Rock, Marin Co. (21 May 1987).
124: Tullock Reservoir Road, Tuolumne Co. (9 May 1988).
125: 10 m S of Copperopolis, Tuolumne Co. (7 May 1988).
201: 5 m SE of Middletown, Napa Co. (20 May 1986).
202: Along Hwy 20 at Lake Co./Calousa Co. line (21 May 1986).
208: Hough Springs, Lake Co. (15 May 1988).

M. micranthus

301: 7 m W of Bartlett Springs, Lake Co. (21 May 1986).
302: 0.7 m S of N entrance to Stevens Ck Park, Santa Cruz Co. (19 May 1986).
305: 14 m W of Willets on road to Fort Bragg, Mendocino Co. (20 May 1988).
306: 1.7 m W on Saratoga Road from intersection with SRs 9 and 85, Santa Cruz Co. (23 May 1988).
307: 2 m N of Laytonville on old Hwy 101 (0.6 m along new Hwy 101), Mendocino Co. (20 May 1988).